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(54) Title: ORGANOMETALLIC LIGANDS FOR THE LOCALIZATION AND QUANTIFICATION OF AMYLOID *IN VIVO* AND *IN VITRO*

(57) Abstract

Novel organometallic compounds for binding amyloid are described. Methods using such compounds for determining by imaging the localization or quantification of amyloid fibrils in a mammal, for diagnosing the degree of progression of Alzheimer's disease in a mammal, for monitoring the response to therapy in a mammal having Alzheimer's disease, for identifying an agent useful for treating Alzheimer's disease, for treating Alzheimer's disease, and for detecting the presence of the infectious form of the prion protein, are also described.

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ORGANOMETALLIC LIGANDS FOR THE LOCALIZATION AND QUANTIFICATION OF AMYLOID *IN VIVO* AND *IN VITRO*

This application claims the benefit of U.S. Provisional Application No. 60/016,599 filed May 8, 1996, and U.S. Provisional Application No. 60/038,999 filed February 25, 1997.

The U.S. Government has a paid-up license in this invention and the right in limited circumstances to require the patent owner to license others on reasonable terms as provided for by the terms of Grant No. AG08470 awarded by the National Institutes of Health, National Institute on Aging.

Field of the Invention

The present invention relates to novel organometallic ligands which interact with amyloid fibrils, and methods for using such ligands for diagnosing, treating and monitoring therapies for Alzheimer's and other diseases.

Background of the Invention

Alzheimer's disease is the most prevalent form of senile dementia. Epidemiological studies suggest that 25-50% of all people in their 80's have Alzheimer's disease. Generally, the first symptom of Alzheimer's disease is memory loss, followed by a decline in reasoning ability and reduced use of speech. Behavioral disorders are also often present. The deterioration appears to be irreversible, and eventually leads to death. There is no effective treatment currently available.

Alzheimer's disease was originally described, and is still diagnosed, based on the presence in the postmortem brain of proteinaceous deposits, known as amyloid plaques, which stain with the dye Congo Red. Amyloid plaques contain a core comprising ordered fibrillar protein aggregates. In Alzheimer's disease, the predominant brain amyloid proteins are β1-42 and its C-terminally truncated relative β1-40.

Determination of amyloid load is typically accomplished by counting Congo Red stained amyloid plaques per microscopic field in brain tissue sections of subjects during autopsies. There is a need for probes which allow localization and quantification of amyloid deposits and which can be used in live persons so as to non-invasively diagnose the presence of Alzheimer's disease and/or monitor the efficacy of different treatments for Alzheimer's disease.

There is also a need for such probes for certain other neurodegenerative diseases which affect various mammals, e.g., the prion diseases, e.g., scrapie, bovine spongiform encephalopathy ("mad cow disease"), and Creutzfeldt-Jacob disease.

Summary of the Invention

It is an object of the invention to provide compounds which can be used *in vivo* for detecting amyloid.

It is yet another object of the invention to provide a safe, effective and easy method for detecting the localization and/or quantification of amyloid fibrils in the body.

It is yet another object of the invention to non-invasively measure amyloid deposits in the body by imaging techniques.

It is yet another object of the invention to use Congo Red or Chrysamine G analogs to carry metals to *in vivo* amyloid plaque for the purpose of imaging.

It is yet another object of the invention to use organometallic compounds which can cross the blood brain barrier to localize and/or quantify amyloid fibrils in the brain.

It is yet another object of the invention to establish the time course of amyloid deposition in the brain of a patient and compare it to the appearance of Alzheimer's disease symptoms in the patient.

It is yet another object of the invention to non-invasively diagnose the presence and/or degree of progression of Alzheimer's disease in a patient.

It is yet another object of the invention to non-invasively monitor treatments for Alzheimer's disease in a patient.

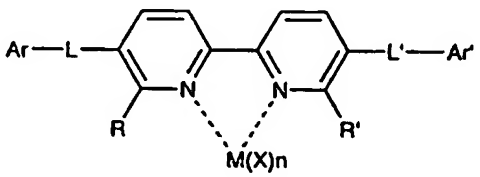
It is yet another object of the invention to treat Alzheimer's disease in a patient with compounds which bind to amyloid fibrils and thereby inhibit their aggregation into amyloid plaques.

It is yet another object of the invention to localize and/or quantify amyloid deposits in the body during autopsies.

It is yet another object of the invention to identify compounds which are useful for treating a disease associated with accumulation of aggregated amyloid.

Still another object of the invention is to provide compounds which can be used for detecting prion protein.

According to the invention amyloid binding compounds are provided. The compounds are of the formula:



(I)

and pharmaceutically acceptable salts thereof,

wherein

R and R^1 are H, N_2H_x (x is 0, 1, 2, 3 or 4), CH_2OH, CH_2NH_2, CH_2SH, o-$C_6H_4CH_2COOH$, $CH_2NHCH_2CH_2SH$, $CH_2P(CH_3)_2$, or $CH_2PCH_2CH_2P(CH_3)_2$, and can be the same or different from each other, and if R or R^1 is not H it can additionally bind or not bind to M, and if R or R^1 is H it cannot bind to M;

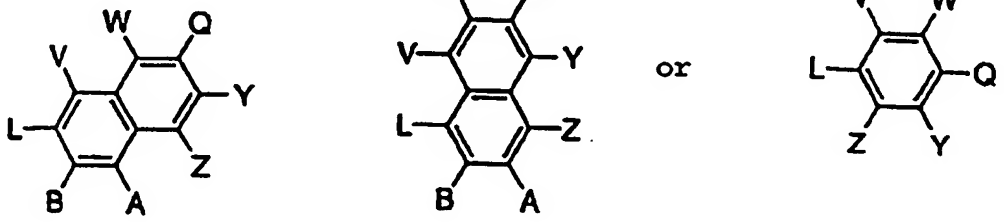
M is ^{99m}Tc, ^{111}In, ^{90}Y, ^{99}Tc, ^{186}Re, Cd, Zn, Co, Cu, Fe, Ni, or oxo forms of these metals;

X is Cl, I, Br, F, $P(R^2)_3$ (R^2 is C_{1-6} hydrocarbon), $P(Ar^2)_3$ (Ar^2 is aryl or substituted aryl), R^3NC (R^3 is C_{1-6} hydrocarbon), Ar^3NC (Ar^3 is aryl or substituted aryl), SR^4 (R^4 is CH_2CH_2SH or C_{1-6} hydrocarbon), or $P(R^5)_2R^6$ (R^5 is C_{1-6} hydrocarbon; R^6 is C_{1-6} hydrocarbon or $CH_2CH_2P(CH_3)_2$, and each X can be the same or different from each other;

n is the number 1, 2, 3 or 4;

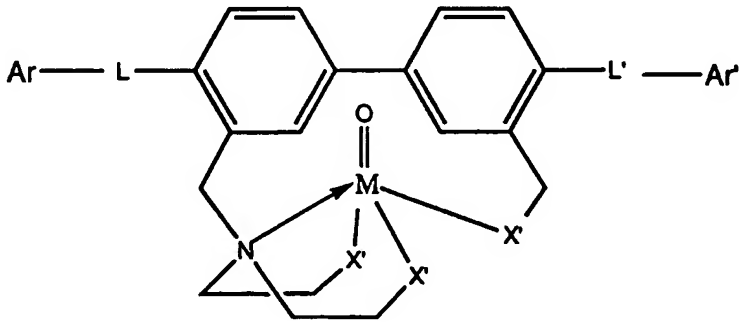
L and L' are -N=N-, -CONH-, -NHCO-, -HN-NH-, or -C=C-, and can be the same or different from each other; and

Ar and Ar' are



and can be the same or different from each other, where each of V, W, Q, Y, Z, A and B is OH, COOH, H, R^5 (R^5 is C_{1-6} hydrocarbon), CO_2R^6 (R^6 is C_{1-6} hydrocarbon), $CONH_2$, CN, NH_2, CH_2NH_2 or SO_3 and can be the same or different from each other.

Another aspect of the invention is an amyloid binding compound of the formula:



(II)

and pharmaceutically acceptable salts thereof,

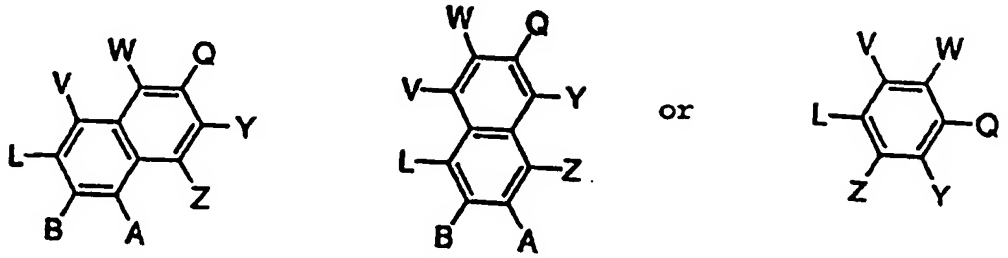
wherein

M is ^{99m}Tc, ^{111}In, ^{90}Y, ^{99}Tc or ^{186}Re;

X' is S, NH or O;

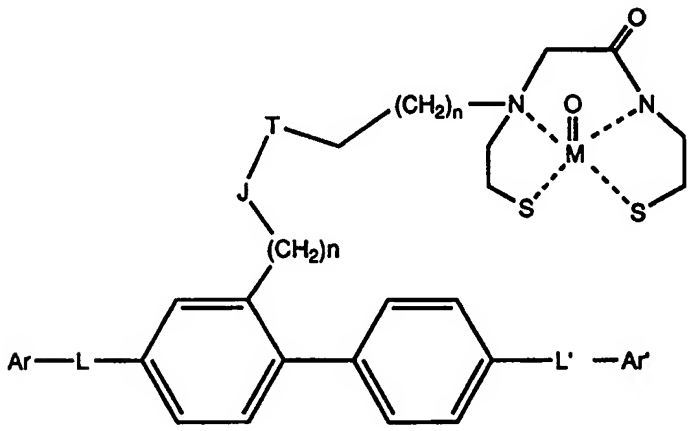
L and L' are -N=N-, -CONH-, -NHCO-, -HN-NH-, or -C=C-, and can be the same or different from each other; and

Ar and Ar' are



and can be the same or different from each other, where each of V, W, Q, Y, Z, A and B is OH, COOH, H, R^5 (R^5 is C_{1-6} hydrocarbon), CO_2R^6 (R^6 is C_{1-6} hydrocarbon), $CONH_2$, CN, NH_2, CH_2NH_2 or SO_3 and can be the same or different from each other.

Another aspect of the invention is an amyloid binding compound of the formula:



(III)

and pharmaceutically acceptable salts thereof,

wherein

J is NH, O or S;

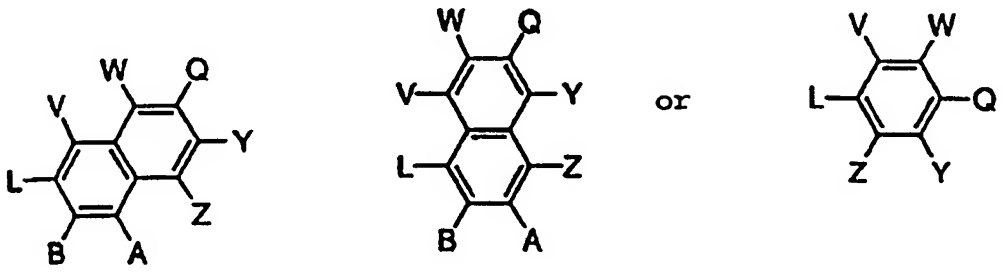
T is CO or CH_2;

n is the number 1, 2, 3, 4, 5 or 6;

M is ^{99m}Tc, ^{111}In, ^{90}Y, ^{99}Tc or ^{186}Re;

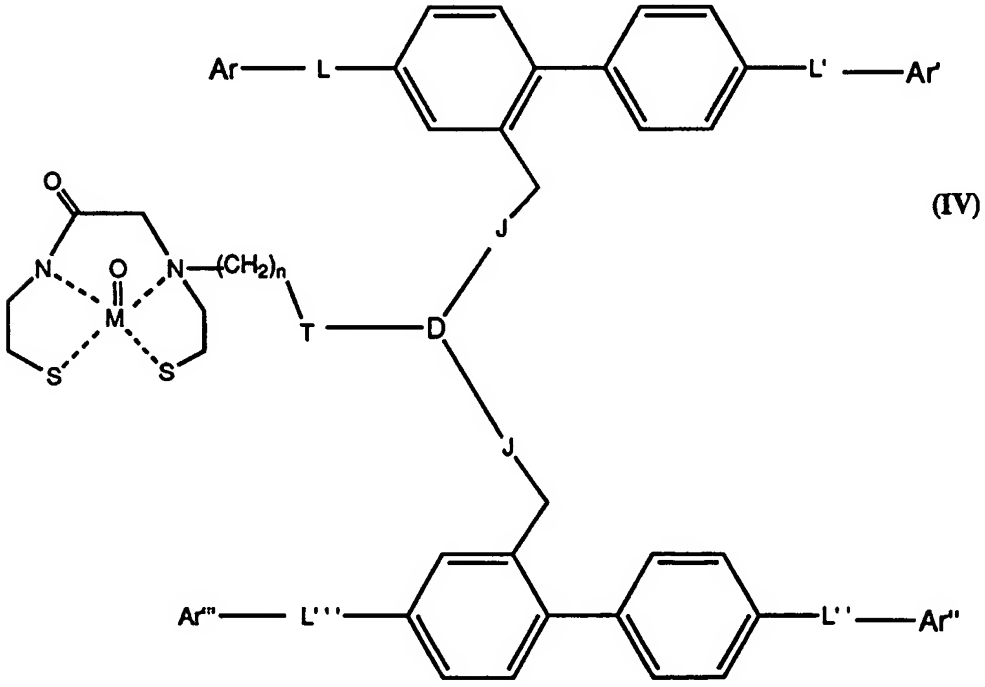
L and L' are -N=N-, -CONH-, -NHCO-, -HN-NH-, or -C=C-, and can be the same or different from each other; and

Ar and Ar' are



and can be the same or different from each other, where each of V, W, Q, Y, Z, A and B is OH, COOH, H, R^5 (R^5 is C_{1-6} hydrocarbon), CO_2R^6 (R^6 is C_{1-6} hydrocarbon), $CONH_2$, CN, NH_2, CH_2NH_2 or SO_3 and can be the same or different from each other.

Another aspect of the invention is an amyloid binding compound of the formula:



(IV)

and pharmaceutically acceptable salts thereof,

wherein

J is NH or S;

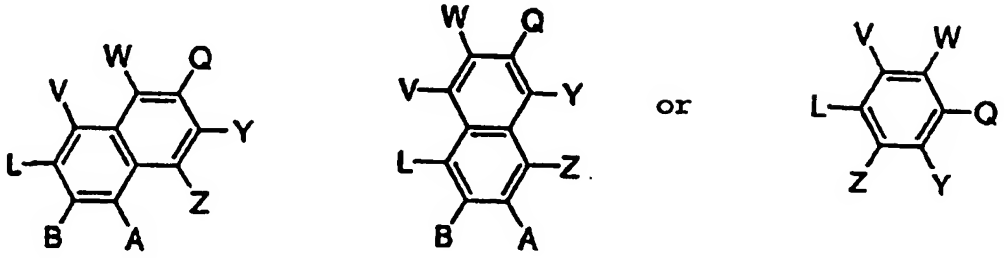
T is CO or CH_2;

n is the number 1, 2, 3, 4, 5 or 6;

M is ^{99m}Tc, ^{111}In, ^{90}Y, ^{99}Tc or ^{186}Re;

L, L', L'' and L''' are -N=N-, -CONH-, -NHCO-, -HN-NH-, or -C=C-, and can be the same or different from each other;

Ar, Ar', Ar'' and Ar''' are



and can be the same or different from each other, where each of V, W, Q, Y, Z, A and B is OH, COOH, H, R^5 (R^5 is C_{1-6} hydrocarbon), CO_2R^6 (R^6 is C_{1-6} hydrocarbon), $CONH_2$, CN, NH_2, CH_2NH_2 or SO_3 and can be the same or different from each other; and

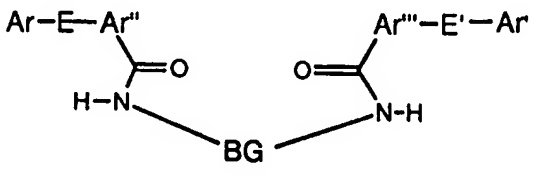
when J is NH and T is CO, then D is a trifunctional linker with two carboxyl groups and one amine group,

when J is NH and T is CH_2, then D is $COCH_2(CH_2S)CH_2CO$,

when J is S and T is CO, then D is $CH_2CH(CH_2NH)CH_2$, and

when J is S and T is CH_2, then D is $CH_2CH(CH_2S)CH_2$.

Another aspect of the invention is an amyloid binding compound of the formula:



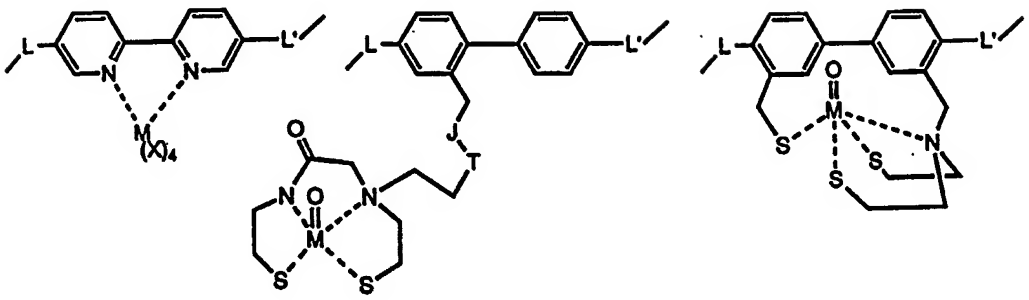
(V)

and pharmaceutically acceptable salts thereof,

wherein

BG is any dicarbonyl or dithiocarbonyl moiety;

E and E' are



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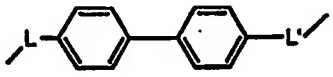


and can be the same or different from each other,

wherein

M is ^{99m}Tc, ^{111}In, ^{90}Y, ^{99}Tc or ^{186}Re;

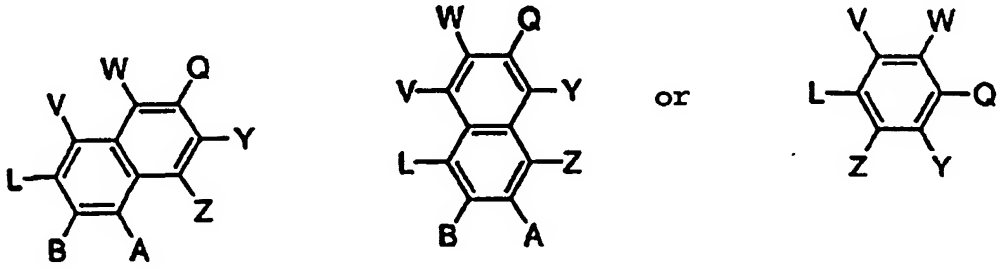
L and L' are -N=N-, -CONH-, -NHCO-, -HN-NH-, or -C=C-, and can be the same or different from each other;

J is NH or S;

T is CO or CH_2;

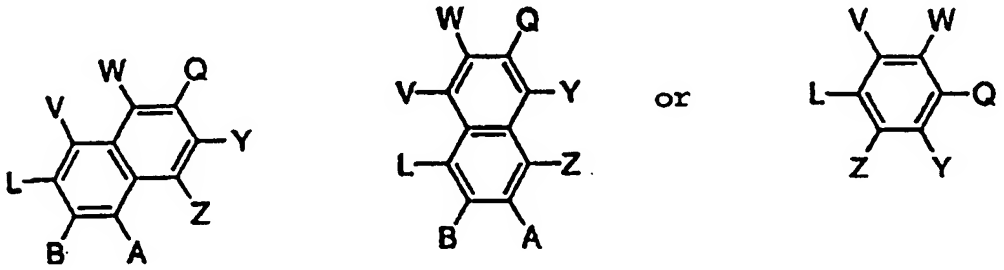
X is Cl, I, Br, F, $P(R^2)_3$ (R^2 is C_{1-6} hydrocarbon), $P(Ar^2)_3$ (Ar^2 is aryl or substituted aryl), R^3NC (R^3 is C_{1-6} hydrocarbon), Ar^3NC (Ar^3 is aryl or substituted aryl), SR^4 (R^4 is CH_2CH_2SH or C_{1-6} hydrocarbon), or $P(R^5)_2R^6$ (R^5 is C_{1-6} hydrocarbon; R^6 is C_{1-6} hydrocarbon or $CH_2CH_2P(CH_3)_2$, and each X can be the same or different from each other;

Ar and Ar' are



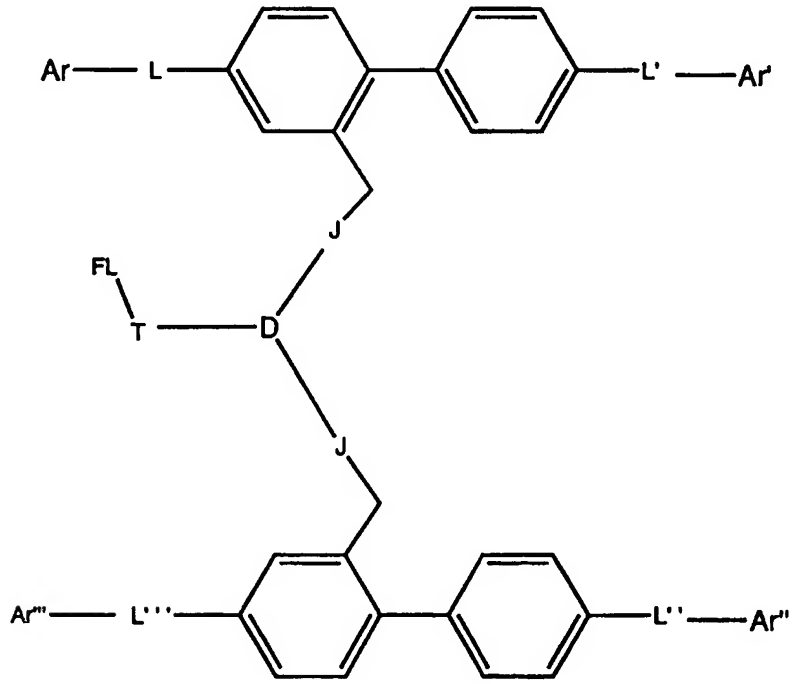
and can be the same or different from each other, where each of V, W, Q, Y, Z, A and B is OH, COOH, H, R^5 (R^5 is C_{1-6} hydrocarbon), CO_2R^6 (R^6 is C_{1-6} hydrocarbon), $CONH_2$, CN, NH_2, CH_2NH_2 or SO_3 and can be the same or different from each other, and

Ar'' and Ar''' are



and can be the same or different from each other, where one of V, W, Q, Y, Z, A and B is COOH and each of the others is OH, COOH, H, R^5 (R^5 is C_{1-6} hydrocarbon), CO_2R^6 (R^6 is C_{1-6} hydrocarbon), $CONH_2$, CN, NH_2, CH_2NH_2 or SO_3 and can be the same or different from each other.

Another aspect of the invention is an amyloid binding compound of the formula:



(IX)

and pharmaceutically acceptable salts thereof,

wherein

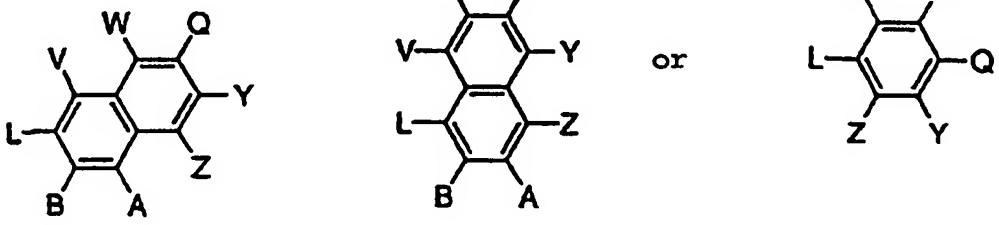
J is NH or S;

T is CO or CO_2;

FL is fluorescein, rhodamine, coumarin or any other fluorescent moiety;

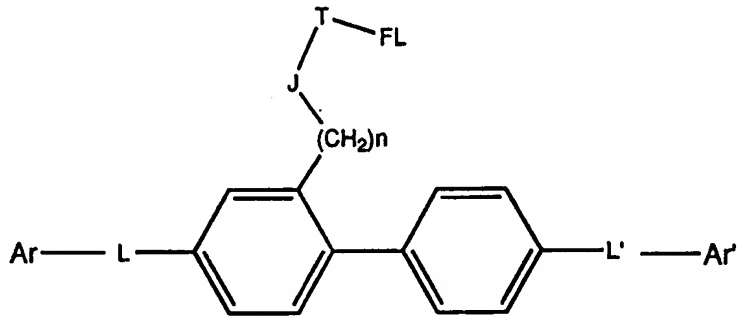
L and L' are -N=N-, -CONH-, -NHCO-, -HN-NH-, or -C=C-, and can be the same or different from each other; and

Ar and Ar' are



and can be the same or different from each other, where each of V, W, Q, Y, Z, A and B is OH, COOH, H, R^5 (R^5 is C_{1-6} hydrocarbon), CO_2R^6 (R^6 is C_{1-6} hydrocarbon), $CONH_2$, CN, NH_2, CH_2NH_2 or SO_3 and can be the same or different from each other.

Another aspect of the invention is an amyloid binding compound of the formula:



(X)

and pharmaceutically acceptable salts thereof,

wherein

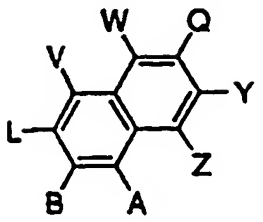
J is NH or S;

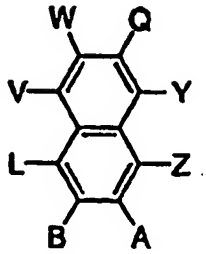
T is CO or CO_2;

FL is fluorescein, rhodamine, coumarin or any other fluorescent moiety;

L and L' are -N=N-, -CONH-, -NHCO-, -HN-NH-, or -C=C-, and can be the same or different from each other; and

Ar and Ar' are





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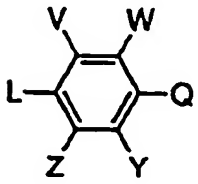


and can be the same or different from each other, where each of V, W, Q, Y, Z, A and B is OH, COOH, H, R^5 (R^5 is C_{1-6} hydrocarbon), CO_2R^6 (R^6 is C_{1-6} hydrocarbon), $CONH_2$, CN, NH_2, CH_2NH_2 or SO_3 and can be the same or different from each other.

Another aspect of the invention is a method for diagnosing the degree of progression of Alzheimer's disease in a mammal. A first mammal having Alzheimer's disease and having brain amyloid fibrils, e.g., protofibrils, type-1 fibrils, type-2 fibrils, neuritic plaque, diffuse amyloid or combinations thereof, is provided. A labeled ligand, e.g., an organometallic ligand, where the label is, e.g., technetium-99m, indium-111, yttrium-90, rhenium-186 or technetium-99, capable of interacting with the amyloid fibrils is also provided. Preferred labeled ligands are compounds of formula I, II, III, IV, V, or pharmaceutically acceptable salts thereof. The labeled ligand is administered to the mammal under conditions which allow the labeled ligand to interact with the amyloid fibrils in the brain so as to result in labeled amyloid fibrils. The localization or quantification of the labeled amyloid fibrils in the mammal is determined by imaging, e.g., radioimaging, magnetic resonance imaging or single photon emission computed tomographic imaging, so as to diagnose the degree of progression of the Alzheimer's disease. In certain embodiments, the localization or quantification of the labeled amyloid fibrils is compared to a standard.

Another aspect of the invention is a method for monitoring the response to a therapy in a mammal having Alzheimer's disease. A mammal having Alzheimer's disease and having brain amyloid fibrils is provided. The mammal is treated with a therapy for Alzheimer's disease. The response of the mammal to the treating step is monitored by determining whether the therapy alters the localization or quantification of the amyloid fibrils in the mammals. In certain embodiments, the determining step comprises providing a labeled ligand, e.g., a compound of formula I, II, III, IV, V, or pharmaceutically acceptable salts thereof, capable of interacting with the amyloid fibrils. The labeled ligand is administered to the mammal under conditions which allow the labeled ligand to interact with

the amyloid fibrils in the brain so as to result in labeled amyloid fibrils. The localization or quantification of the labeled amyloid fibrils in the mammal is determined by imaging.

Another aspect of the invention is a method for evaluating the ability of an agent to alter the localization or quantification of brain amyloid fibrils in a mammal. A mammal having brain amyloid fibrils is provided. An agent is provided. The agent is administered to the mammal and it is determined whether the agent alters the localization or quantification of the brain amyloid fibrils in the mammal.

Another aspect of the invention is a method for identifying an agent useful for treating a mammal having a disease associated with aggregated amyloid. A mammal having such a disease and having amyloid fibrils is provided. An agent is provided and administered to the mammal. It is determined if the agent alters the localization or quantification of the amyloid fibrils in the mammal. An alteration in the localization or quantification which results in a localization or quantification more similar to that of a mammal which does not have the disease is correlated with the agent being useful for treating the mammal having the disease. The invention also includes the agent obtainable by this method.

Another aspect of the invention is a method for determining the localization or quantification of amyloid fibrils in a mammal. A mammal having amyloid fibrils, e.g, in the brain, pancreas, vasculature, spleen, liver, kidneys, adrenals, lymph nodes, muscle, cardiovascular system, skin, or any combination thereof, is provided. An organometallic ligand capable of interacting with the amyloid fibrils is provided, e.g., a compound of formula **I**, **II**, **III**, **IV**, **V**, or pharmaceutically acceptable salts thereof. The organometallic ligand is administered to the mammal under conditions which allow the organometallic ligand to interact with the amyloid fibrils so as to result in organometallic ligand-amyloid fibril complexes. The localization or quantification of the complexes is determined in the mammal, e.g., by imaging. In certain embodiments, the administering and determining steps are repeated after a time interval so as to establish a time course for the localization or quantification of the complexes in the mammal. In other embodiments, the mammal is deceased, and the administering step is, e.g., to the postmortem brain or a portion thereof.

Another aspect of the invention is a method for treating Alzheimer's disease in a mammal. A mammal having Alzheimer's disease is provided. The mammal has non-aggregated amyloid proteins or aggregated amyloid proteins, or combinations thereof. An organometallic ligand capable of interacting with the non-aggregated amyloid proteins, or with the aggregated amyloid proteins, or with both of the amyloid proteins, is provided, e.g.,

a compound of formula I, II, III, IV, V, or pharmaceutically acceptable salts thereof. A therapeutically effective amount of the organometallic ligand is administered to the mammal under conditions which allow the organometallic ligand to interact with the non-aggregated amyloid proteins so as to inhibit aggregation of the amyloid proteins, or with the aggregated amyloid proteins, or with both of the amyloid proteins, such that treatment of the Alzheimer's disease occurs.

Another aspect of the invention is a pharmaceutical composition for treating Alzheimer's disease in a mammal comprising a therapeutically effective amount of an organometallic ligand, e.g., a compound of formula I, II, III, IV, V, or pharmaceutically acceptable salts thereof, the ligand being able to interact with amyloid proteins in a mammal in need of treatment for Alzheimer's disease, and a pharmaceutically acceptable carrier.

Another aspect of the invention is a method for determining the localization or quantification of amyloid fibrils in a deceased mammal. A deceased mammal or a portion thereof having amyloid fibrils is provided. An organometallic ligand, e.g., a compound of formula I, II, III, IV, V, or pharmaceutically acceptable salts thereof, capable of interacting with the amyloid fibrils is provided. The organometallic ligand is administered to the mammal or portion thereof under conditions which allow the organometallic ligand to interact with the amyloid fibrils so as to result in organometallic ligand-amyloid fibril complexes. The localization or quantification of the complexes in the mammal or portion thereof is determined, e.g., by autoradiography, SPECT or PET imaging.

Another aspect of the invention is a method for detecting the presence of aggregated prion protein in a mammal. A mammal is provided. Bodily fluid or tissue, e.g., lymph, blood or urine, obtained from the mammal is provided. A labeled ligand capable of interacting with aggregated prion protein is provided, e.g., a compound of formula I, II, III, IV, V, or pharmaceutically acceptable salts thereof. The bodily fluid or tissue is contacted *in vitro* with the labeled ligand under conditions which allow the labeled ligand to interact with the aggregated prion protein if the aggregated prion protein is present in the bodily fluid or tissue, so as to result in labeled aggregated prion protein. The presence or absence of the labeled aggregated prion protein in the bodily fluid or tissue is determined. In certain embodiments, the mammal has a prion disease, e.g., scrapie, bovine spongiform encephalopathy or Creutzfeldt-Jacob disease.

Another aspect of the invention is a method for detecting the presence of aggregated prion protein in a mammal. A mammal is provided. A labeled ligand capable of interacting

with aggregated prion protein is provided, e.g., a compound of formula I, II, III, IV, V, or pharmaceutically acceptable salts thereof. The labeled ligand is administered to the mammal under conditions which allow the labeled ligand to interact with the aggregated prion protein if the aggregated prion protein is present in the mammal, so as to result in labeled aggregated prion protein. The presence or absence of the labeled aggregated prion protein is determined in the mammal by imaging.

Another aspect of the invention is a method for determining the presence of aggregated intracellular β-amyloid. Cells having β-amyloid are provided. A fluorescent ligand capable of interacting with aggregated β-amyloid, e.g., a compound of formula IX or X, is provided. The cells are contacted with the fluorescent ligand under conditions which allow the fluorescent ligand to interact with aggregated β-amyloid if it is present so as to result in fluorescent-labeled aggregated β-amyloid. The presence or absence of a fluorescent signal is determined. The presence of a fluorescent signal indicates the presence of aggregated intracellular β-amyloid.

Another aspect of the invention is a method for identifying an agent useful for treating a mammal for a disease characterized by aggregated intracellular β-amyloid. Cells having β-amyloid are provided. An agent is provided. A fluorescent ligand capable of interacting with β-amyloid fibrils, e.g., a compound of formula IX or X, is provided. The cells are contacted with the agent to form a mixture under conditions which allow aggregation of the β-amyloid if the agent was not present. The mixture is contacted with the fluorescent ligand under conditions which allow the fluorescent ligand to interact with β-amyloid fibrils if they are present so as to result in fluorescent-labeled β-amyloid fibrils. It is determined if the agent inhibits aggregation of the β-amyloid. The presence of a fluorescent signal indicates the presence of β-amyloid fibrils and therefore minimal or no inhibition by the agent. The absence of a fluorescent signal indicates the absence of β-amyloid fibrils and therefore inhibition by the agent. This inhibition is correlated with the agent being useful for treating a mammal for a disease characterized by aggregated intracellular β-amyloid.

Another aspect of the invention is a method for identifying a labeled ligand which selectively binds to one type of β-amyloid fibril. A labeled compound is provided, e.g., a compound of formula I, II, III, IV, V, or pharmaceutically acceptable salts thereof. First β-amyloid fibrils are provided, and second β-amyloid are provided. The labeled compound is contaced with the first β-amyloid fibrils under conditions which allow the labeled ligand to interact with the first β-amyloid fibrils. It is determined if the labeled compound binds to the

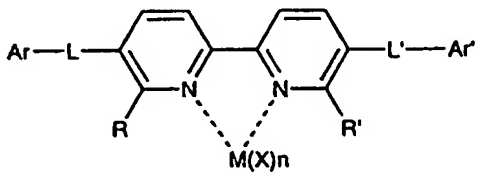
first β-amyloid fibrils. If the labeled compound does not bind to the first β-amyloid fibrils, then the labeled compound is contacted with the second β-amyloid fibrils under conditions which allow the labeled compound to interact with the second β-amyloid fibrils. It is determined if the labeled compound binds to the second β-amyloid fibrils, binding being correlated with a labeled ligand which selectively binds to the second β-amyloid fibrils as compared to the first β-amyloid fibrils. The invention also includes the labeled ligand obtainable from this method.

Another aspect of the invention is a method for identifying a labeled ligand which binds to one or more amyloid proteins, e.g., β-amyloid, Islet amyloid polypeptide, Ig light chain, transthyretin, lysozyme, or β_2-microglobulin, using, e.g., a labeled compound of formula I, II, III, IV, V, or pharmaceutically acceptable salts thereof. The invention also includes the labeled ligand obtainable from this method.

The above and other objects, features and advantages of the present invention will be better understood from the following specification.

Detailed Description

This invention provides an amyloid binding compound of the formula:



(I)

and pharmaceutically acceptable salts thereof,

wherein

R and R^1 are H, N_2H_x (x is 0, 1, 2, 3 or 4), CH_2OH, CH_2NH_2, CH_2SH, o-$C_6H_4CH_2COOH$, $CH_2NHCH_2CH_2SH$, $CH_2P(CH_3)_2$, or $CH_2PCH_2CH_2P(CH_3)_2$, and can be the same or different from each other, and if R or R^1 is not H it can additionally bind or not bind to M, and if R or R^1 is H it cannot bind to M;

M is ^{99m}Tc, ^{111}In, ^{90}Y, ^{99}Tc, ^{186}Re, Cd, Zn, Co, Cu, Fe, Ni, or oxo forms of these metals;

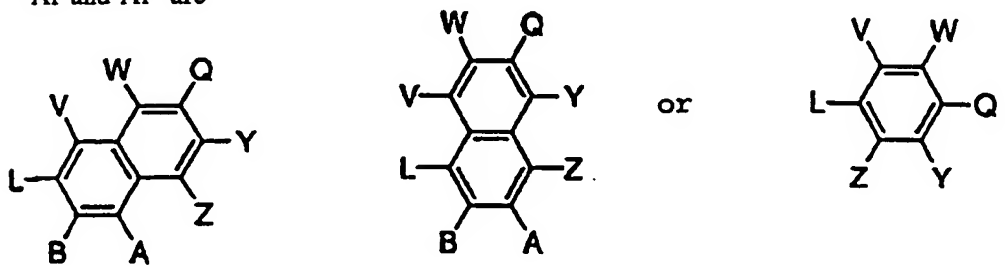
X is Cl, I, Br, F, $P(R^2)_3$ (R^2 is C_{1-6} hydrocarbon), $P(Ar^2)_3$ (Ar^2 is aryl or substituted aryl), R^3NC (R^3 is C_{1-6} hydrocarbon), Ar^3NC (Ar^3 is aryl or substituted aryl), SR^4 (R^4 is CH_2CH_2SH or C_{1-6} hydrocarbon), or $P(R^5)_2R^6$ (R^5 is C_{1-6} hydrocarbon; R^6 is C_{1-6} hydrocarbon

or $CH_2CH_2P(CH_3)_2$, and each X can be the same or different from each other;

n is the number 1, 2, 3 or 4;

L and L' are -N=N-, -CONH-, -NHCO-, -HN-NH-, or -C=C-, and can be the same or different from each other; and

Ar and Ar' are



and can be the same or different from each other, where each of V, W, Q, Y, Z, A and B is OH, COOH, H, R^5 (R^5 is C_{1-6} hydrocarbon), CO_2R^6 (R^6 is C_{1-6} hydrocarbon), $CONH_2$, CN, NH_2, CH_2NH_2 or SO_3 and can be the same or different from each other.

These compounds are referred to herein as BIPY compounds. The metals, M, can be radioactive, e.g., ^{99m}Tc, ^{111}In, ^{90}Y, ^{99}Tc or ^{186}Re, or non-radioactive, e.g., Cd, Zn, Co, Cu, Fe or Ni. Preferably, the radioactive metal is ^{99m}Tc. The oxo forms of the metals, e.g., Re=O or Tc=O, are preferred. The oxo forms are generally more stable and are charge neutral.

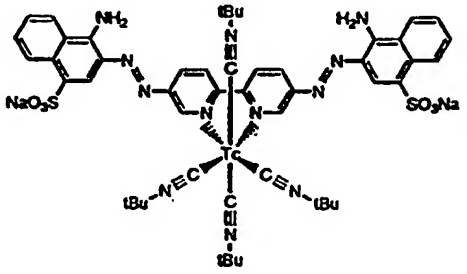
In certain embodiments, if the R or R^1 groups are any group except H, each or both can, but do not have to, additionally bind to M. See, e.g., compounds **15**, **28**, **35**, **45**, **54** and **66**.

When the X moiety is $P(R^2)_3$, the R^2 can be any C_{1-6} hydrocarbon, e.g., alkyl or substituted alkyl group, e.g., methyl or ethyl or t-butyl or, e.g., any aryl or substituted aryl group, e.g, phenyl, paramethoxyphenyl or napthyl. When the X moiety is $P(Ar^2)_3$, the Ar^2 can be any aryl or substituted aryl group, e.g., phenyl, paramethoxyphenyl or napthyl. When the X moiety is an isonitrile, R^3NC, the R^3 can be any C_{1-6} hydrocarbon, e.g., alkyl or substituted alkyl group, e.g., methyl, ethyl, or t-butyl, or, e.g., any aryl or substituted aryl group, e.g., phenyl, paramethoxyphenyl or napthyl. When the X moiety is Ar^3NC, the Ar^3 can be any aryl or substituted aryl group, e.g., phenyl, paramethoxyphenyl or napthyl. When the X moiety is SR^4, the R^4 can be any C_{1-6} hydrocarbon, e.g., alkyl or substituted alkyl group, e.g., methyl, ethyl, or t-butyl, or, e.g., any aryl or substituted aryl group, e.g., phenyl, paramethoxyphenyl or napthyl, or the R^4 can be CH_2CH_2SH. When the X moiety is

$P(R^5)_2R^6$, the R^5 can be any C_{1-6} hydrocarbon, e.g., alkyl or substituted alkyl group, e.g., methyl, ethyl or t-butyl, or, e.g., any aryl or substituted aryl group, e.g., phenyl, paramethoxyphenyl or napthyl, and R^6 can be $CH_2CH_2P(CH_3)_2$ or any C_{1-6} hydrocarbon, e.g., alkyl or substituted alkyl group, e.g., methyl, ethyl or t-butyl, or, e.g., any aryl or substituted aryl group, e.g., phenyl, paramethoxyphenyl or napthyl. n is the number of X moieties present, which is the number required to satisfy the valence requirements of the metal, and can be 1, 2, 3 or 4.

Preferred compounds of formula I have the formulas:

3



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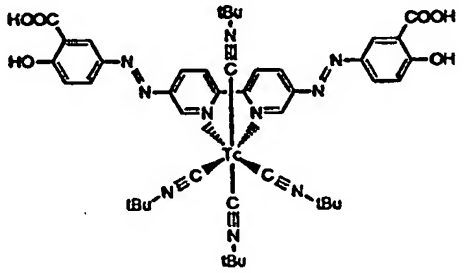
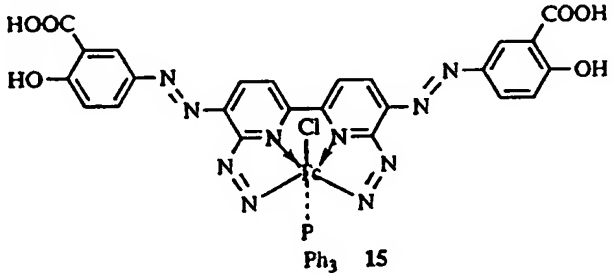


Other preferred compounds of formula I have the formulas:



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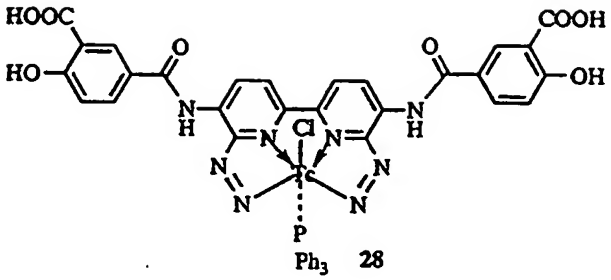
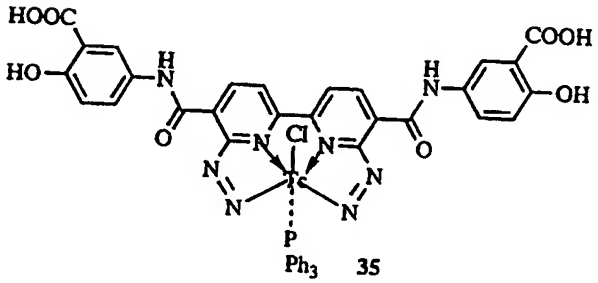


28



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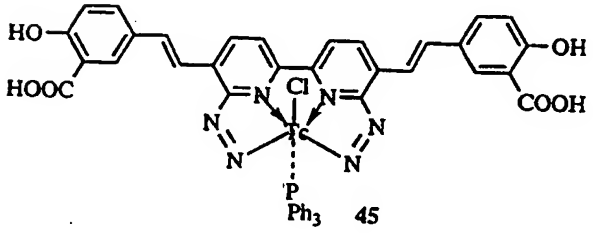

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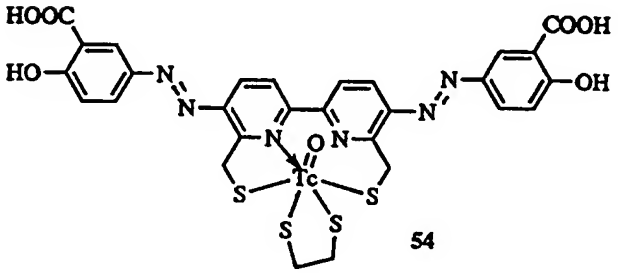
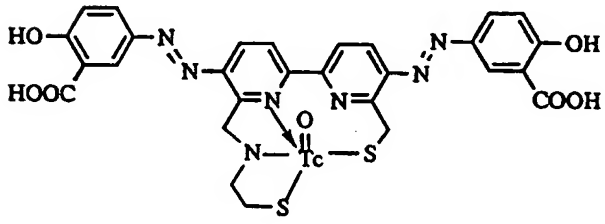
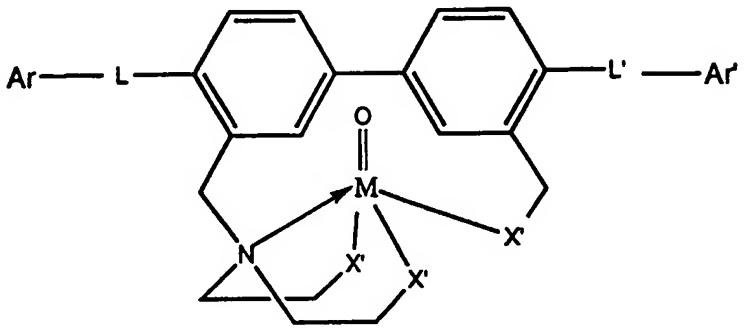


54



66

This invention also provides an amyloid binding compound of the formula:



(II)

and pharmaceutically acceptable salts thereof,

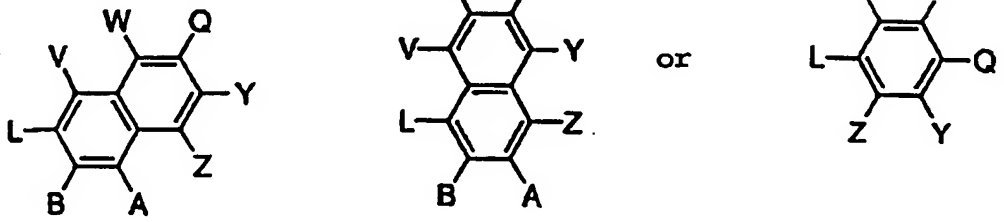
wherein

M is ^{99m}Tc, ^{111}In, ^{90}Y, ^{99}Tc or ^{186}Re;

X' is S, NH or O;

L and L' are -N=N-, -CONH-, -NHCO-, -HN-NH-, or -C=C-, and can be the same or different from each other; and

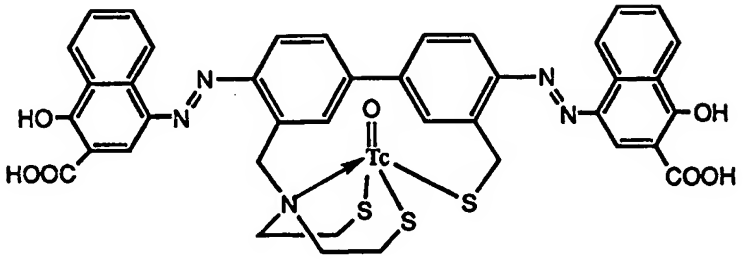
Ar and Ar' are



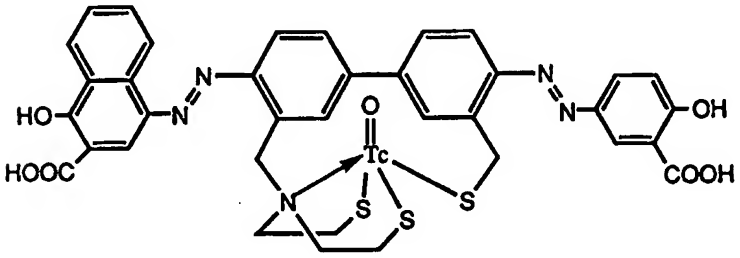
and can be the same or different from each other, where each of V, W, Q, Y, Z, A and B is OH, COOH, H, R^5 (R^5 is C_{1-6} hydrocarbon), CO_2R^6 (R^6 is C_{1-6} hydrocarbon), $CONH_2$, CN, NH_2, CH_2NH_2 or SO_3 and can be the same or different from each other.

Compounds of formula II, referred to herein as NX_3 compounds, can be used, e.g., in SPECT imaging of amyloid, described herein. An advantage of compounds of formula II is that the metal complex is uncharged and therefore can pass more easily through the blood brain barrier when administered to mammals. The uncharged ^{99m}Tc complex is easier to prepare than a charged complex (as, e.g., compounds of formula I), a consideration which influences the practicality of such a reagent for widespread SPECT imaging.

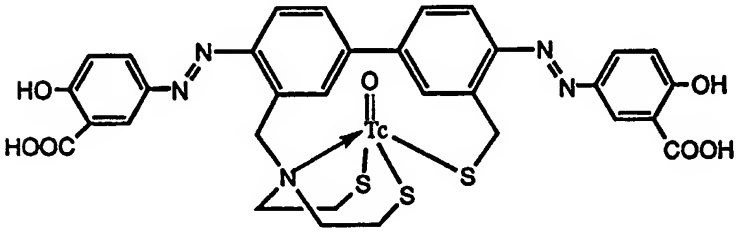
Preferred compounds of formula II have the formulas:



140

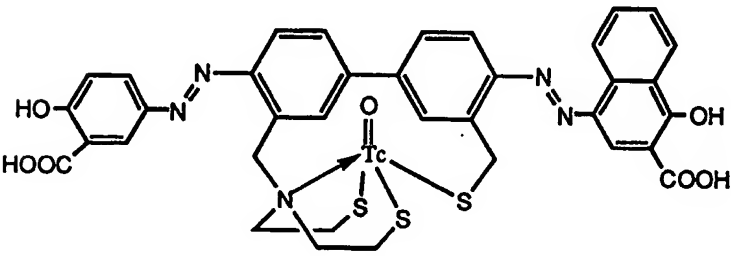


141



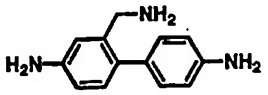
142

and



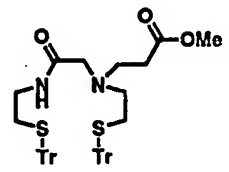
143

The invention also provides a compound of the formula



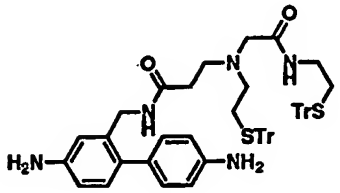
79

The invention also provided a compound of the formula



82

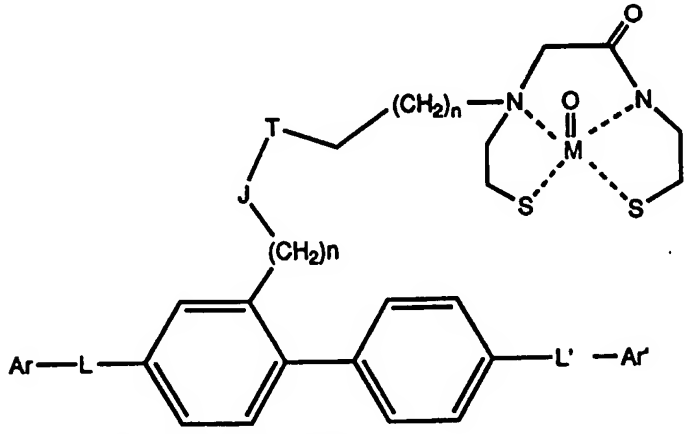
The invention also provides a compound of the formula



83

Each of the above three compounds can be used to synthesize the amyloid binding compounds of formula II discussed above. In addition, compounds 79 and 82 allow the synthesis of N_2S_2 dimers (formula IV) and a type of head-to-tail dimer (formula VIII). Compound 79 also allows the synthesis of fluorescent probes (formulas IX and X).

The invention also provides an amyloid binding compound of the formula



(III)

and pharmaceutically acceptable salts thereof,

wherein

J is NH, O or S;

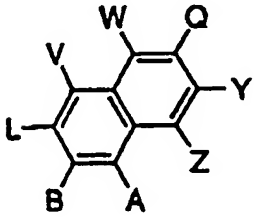
T is CO or CH_2;

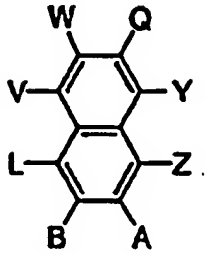
n is the number 1, 2, 3, 4, 5 or 6;

M is ^{99m}Tc, ^{111}In, ^{90}Y, ^{99}Tc or ^{186}Re;

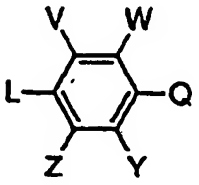
L and L' are -N=N-, -CONH-, -NHCO-, -HN-NH-, or -C=C-, and can be the same or different from each other; and

Ar and Ar' are





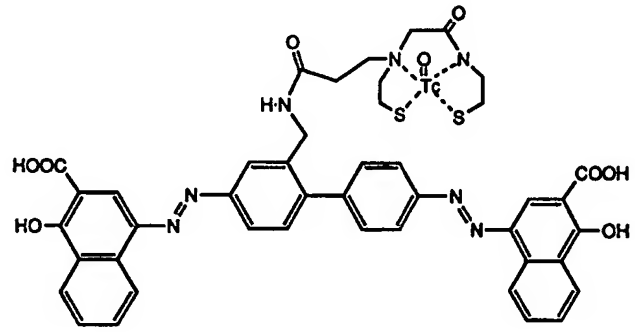
or



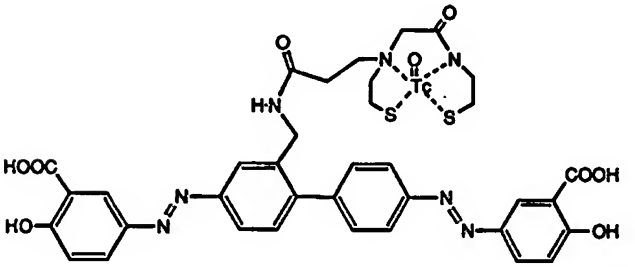
and can be the same or different from each other, where each of V, W, Q, Y, Z, A and B is OH, COOH, H, R^5 (R^5 is C_{1-6} hydrocarbon), CO_2R^6 (R^6 is C_{1-6} hydrocarbon), $CONH_2$, CN, NH_2, CH_2NH_2 or SO_3 and can be the same or different from each other.

Compounds of formula III, referred to herein as N_2S_2 compounds, can be used, e.g., in SPECT imaging of amyloid, described herein. An advantage of compounds of formula III is that the metal complex is uncharged and therefore can pass more easily through the blood brain barrier when administered to mammals. The uncharged metal complexes are easier to prepare than a charged complex (as, e.g., compounds of formula I), a consideration which influences the practicality of such a reagent for widespread SPECT imaging.

Preferred compounds of formula III have the formulas:



144



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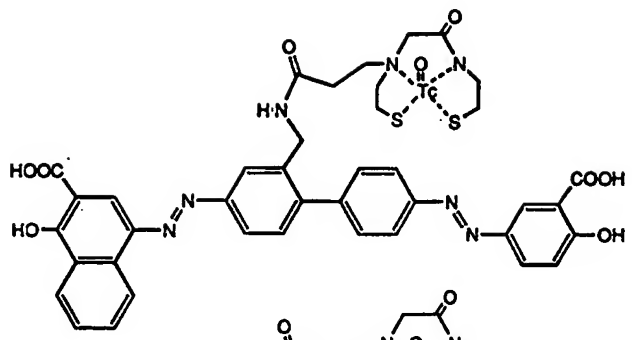
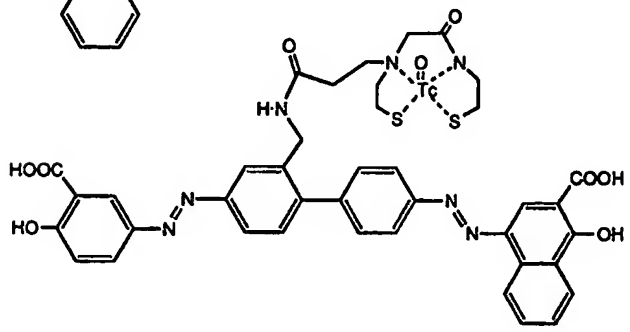
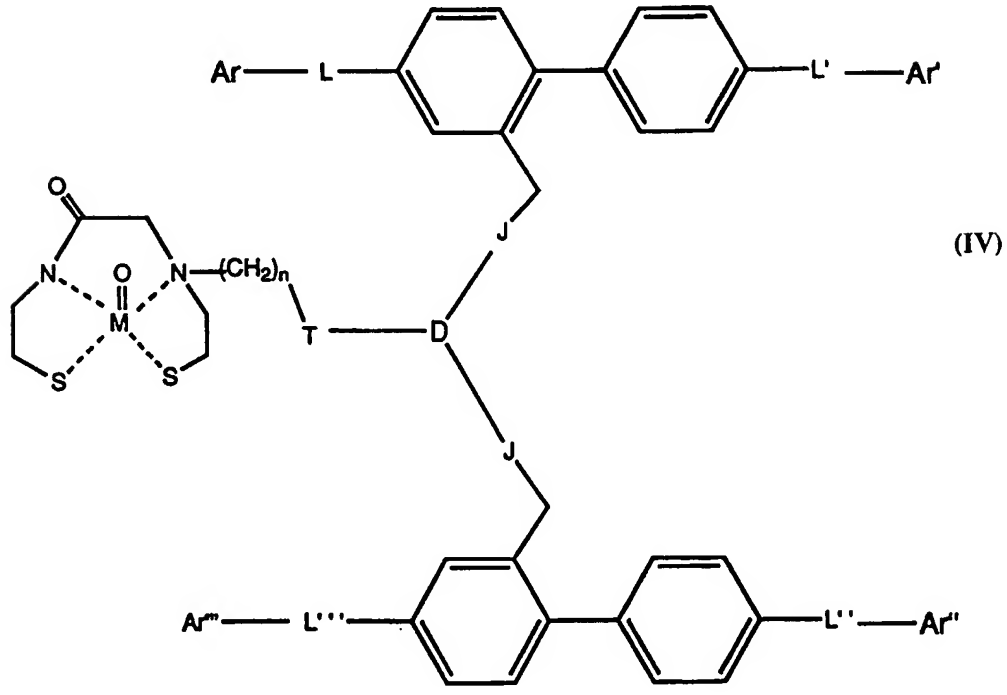


146 and



147

The invention also provides an amyloid binding compound of the formula



(IV)

and pharmaceutically acceptable salts thereof,

wherein

J is NH or S;

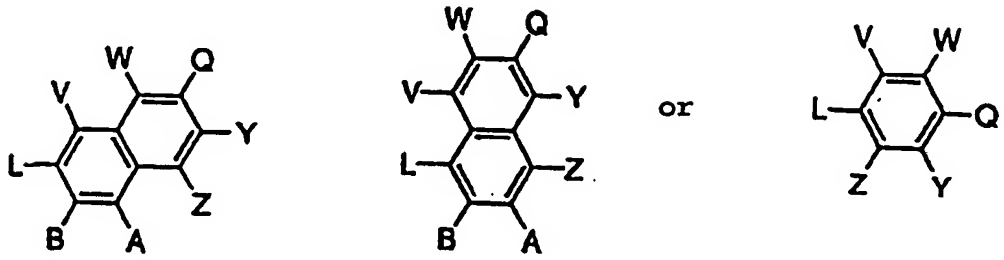
T is CO or CH_2;

n is the number 1, 2, 3, 4, 5 or 6;

M is ^{99m}Tc, ^{111}In, ^{90}Y, ^{99}Tc or ^{186}Re;

L, L', L'' and L''' are -N=N-, -CONH-, -NHCO-, -HN-NH-, or -C=C-, and can be the same or different from each other;

Ar, Ar', Ar'' and Ar''' are



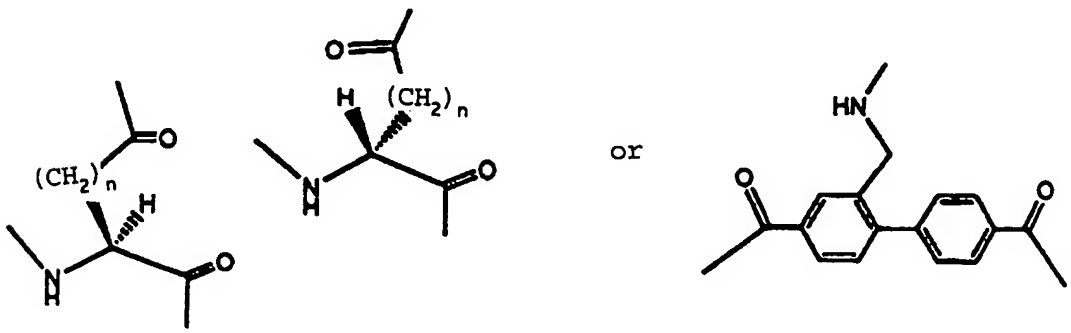
and can be the same or different from each other, where each of V, W, Q, Y, Z, A and B is OH, COOH, H, R^5 (R^5 is C_{1-6} hydrocarbon), CO_2R^6 (R^6 is C_{1-6} hydrocarbon), $CONH_2$, CN, NH_2, CH_2NH_2 or SO_3 and can be the same or different from each other; and

when J is NH and T is CO, then D is a trifunctional linker with two carboxyl groups and one amine group; and

when J is S and T is CO, then D is $CH_2CH(CH_2NH)CH_2$; and

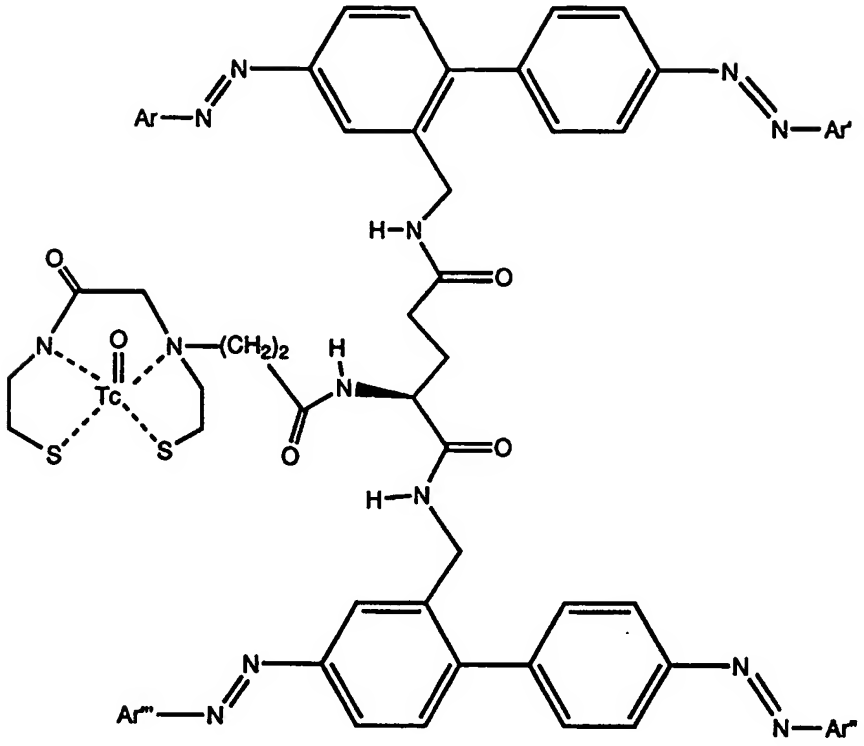
when J is S and T is CH_2, then D is $CH_2CH(CH_2S)CH_2$.

In certain embodiments, when J is NH and T is CO, then D is



and n is the number 1 or 2.

Preferred compounds of formula IV have the formula:



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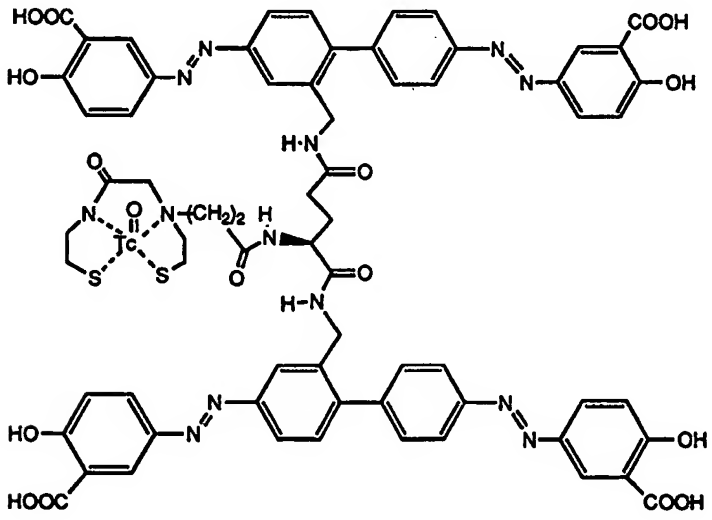
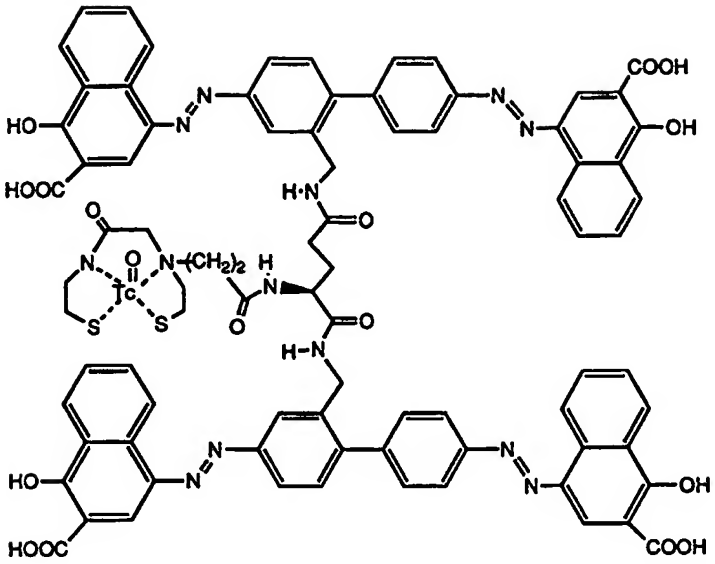
Examples of compounds of formula IV include:



149

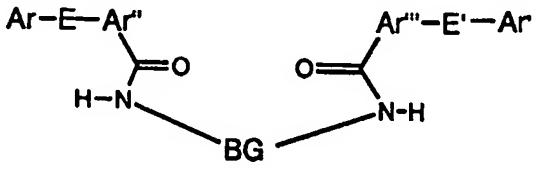
and



Compounds of formula IV (N_2S_2 dimers with a trifunctional linker) can be synthesized, e.g., as described in Example 26 or by combinatorial synthesis of libraries as described in Example 31.

Compounds of formula IV can be used, e.g., in SPECT imaging of amyloid, described herein. An advantage of these dimeric compounds is that they can have increased affinity for binding amyloid as compared to the related monomeric compounds.

The invention also provides an amyloid binding compound of the formula



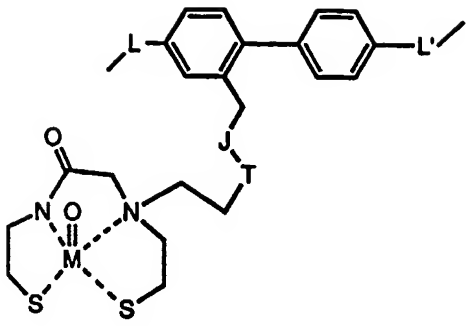
(V)

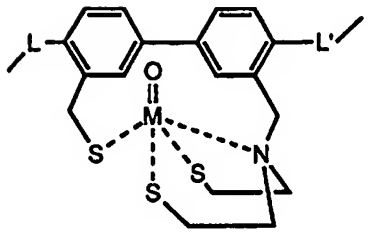
and pharmaceutically acceptable salts thereof,

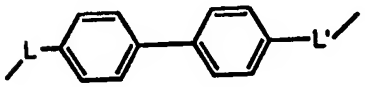
wherein

BG is any dicarbonyl or dithiocarbonyl moiety;

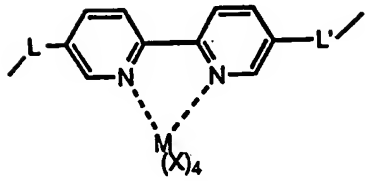
E and E' are







or



and can be the same or different from each other, wherein

M is ^{99m}Tc, ^{111}In, ^{90}Y, ^{99}Tc or ^{186}Re;

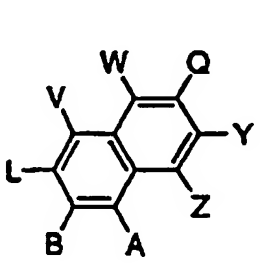
L and L' are -N=N-, -CONH-, -NHCO-, -HN-NH-, or -C=C-, and can be the same or different from each other; and

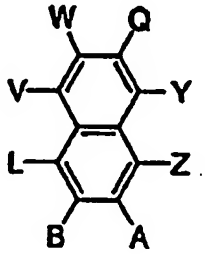
J is NH or S;

T is CO or CH_2;

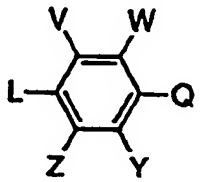
X is Cl, I, Br, F, $P(R^2)_3$ (R^2 is C_{1-6} hydrocarbon), $P(Ar^2)_3$ (Ar^2 is aryl or substituted aryl), R^3NC (R^3 is C_{1-6} hydrocarbon), Ar^3NC (Ar^3 is aryl or substituted aryl), SR^4 (R^4 is CH_2CH_2SH or C_{1-6} hydrocarbon), or $P(R^5)_2R^6$ (R^5 is C_{1-6} hydrocarbon; R^6 is C_{1-6} hydrocarbon or $CH_2CH_2P(CH_3)_2$, and each X can be the same or different from each other; and

Ar and Ar' are



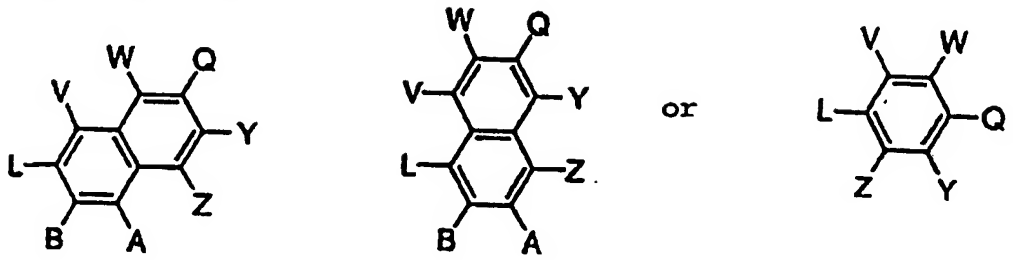


or



and can be the same or different from each other, where each of V, W, Q, Y, Z, A and B is OH, COOH, H, R^5 (R^5 is C_{1-6} hydrocarbon), CO_2R^6 (R^6 is C_{1-6} hydrocarbon), $CONH_2$, CN, NH_2, CH_2NH_2 or SO_3 and can be the same or different from each other, and

Ar'' and Ar''' are

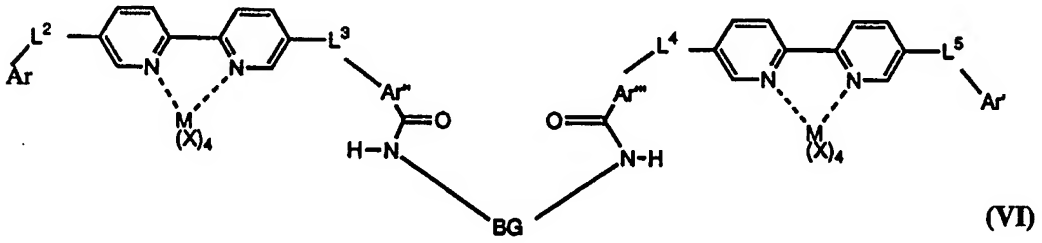


and can be the same or different from each other, where one of V, W, Q, Y, Z, A and B is COOH and each of the others is OH, COOH, H, R^5 (R^5 is C_{1-6} hydrocarbon), CO_2R^6 (R^6 is C_{1-6} hydrocarbon), $CONH_2$, CN, NH_2, CH_2NH_2 or SO_3 and can be the same or different from each other.

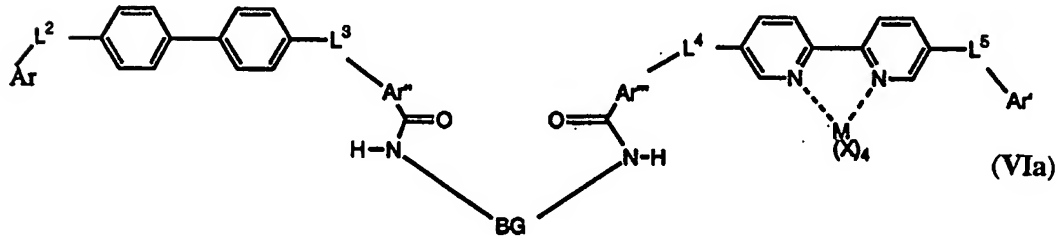
In certain preferred embodiments, BG is $CO(CH_2)_nCO$, $CS(CH_2)_nCS$, $COCH(NH_2)(CH_2)_2CO$ or $COCH(NH_2)CH_2CO$, and n is the number 1-6.

The dimeric compounds of formula V can be synthesized, e.g., directly or by combinatorial synthesis of libraries, as described, e.g., in Example 31. Compounds of formula V can be used, e.g., in SPECT imaging of amyloid, described herein. An advantage of these dimeric compounds is that they can have increased affinity for binding amyloid as compared to the related monomeric compounds.

Preferred compounds of formula V (BIPY dimers) have the formulas:

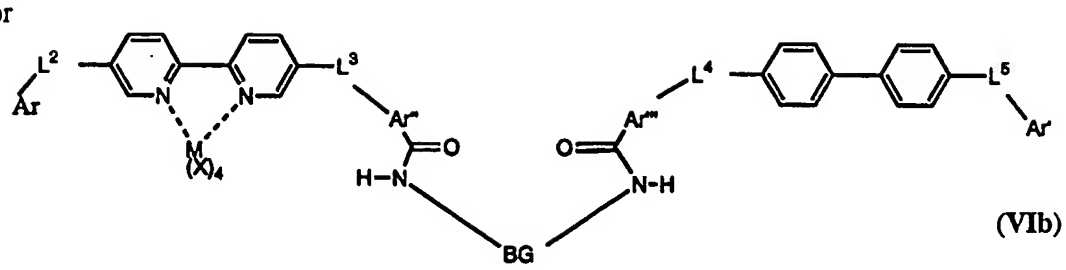


(VI)



(VIa)

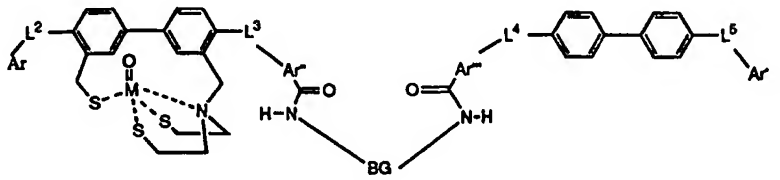
or



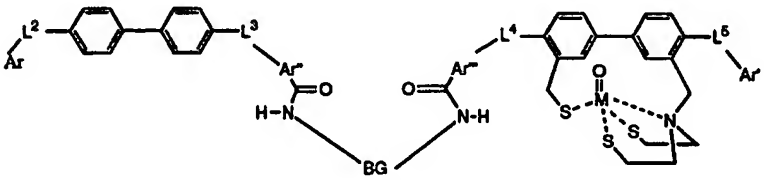
(VIb)

In the above, L^2, L^3, L^4 and L^5 are -N=N-, -CONH-, -NHCO-, -HN-NH-, or -C=C-, and can be the same or different from each other.

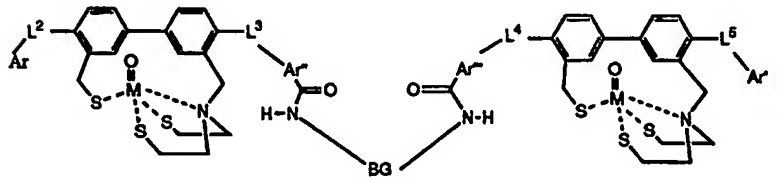
Other preferred compounds of formula V (NX_3 dimers) have the formulas:



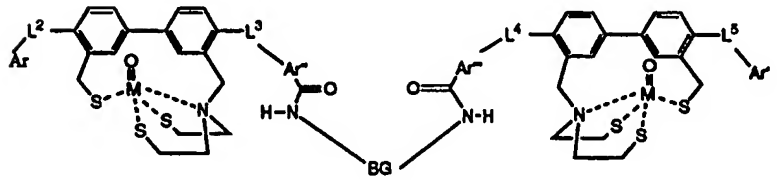
(VII)



(VIIa)

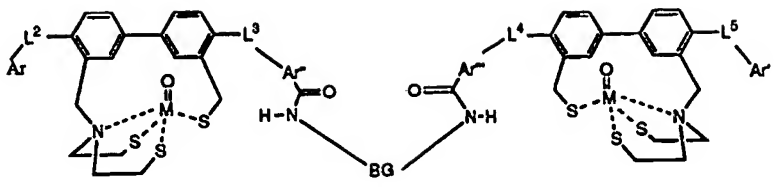


(VIIb)



(VIIc)

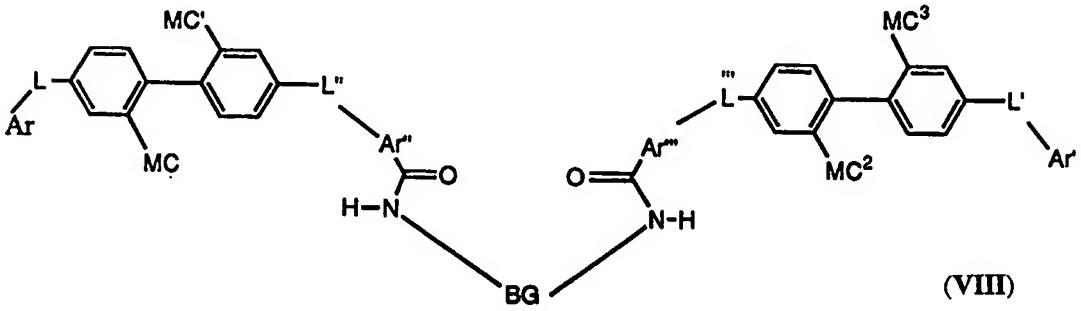
or



(VIId)

wherein L^2, L^3, L^4 and L^5 are -N=N-, -CONH-, -NHCO-, -HN-NH-, or -C=C-, and can be the same or different from each other.

Other preferred compounds of formula V (N_2S_2 dimers) have the formula:

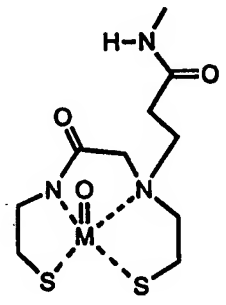


(VIII)

wherein

L^2, L^3, L^4 and L^5 are -N=N-, -CONH-, -NHCO-, -HN-NH-, or -C=C-, and can be the same or different from each other; and

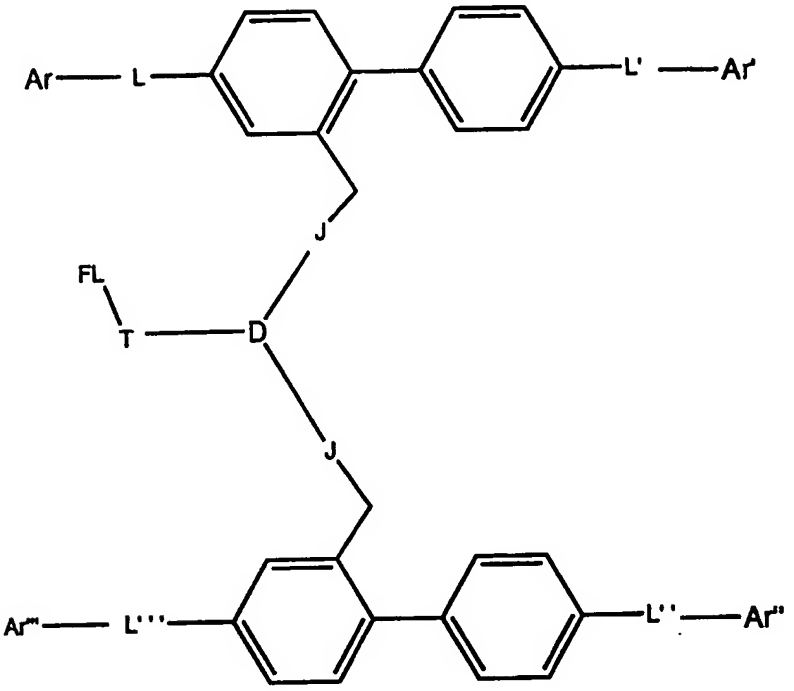
MC, MC^1, MC^2 and MC^3 are



or H

where any one of MC, MC^1, MC^2 or MC^3 is a metal binding group and the others are H, or where MC or MC^1 is H and MC^2 or MC^3 is H and the others are a metal binding group.

The invention also provides an amyloid binding compound of the formula



(IX)

and pharmaceutically acceptable salts thereof,

wherein

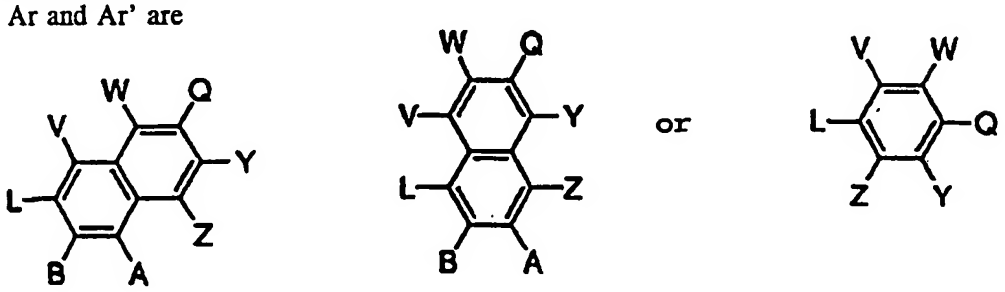
J is NH or S;

T is CO or CO_2;

FL is fluorescein, rhodamine, coumarin, or any other fluorescent moiety;

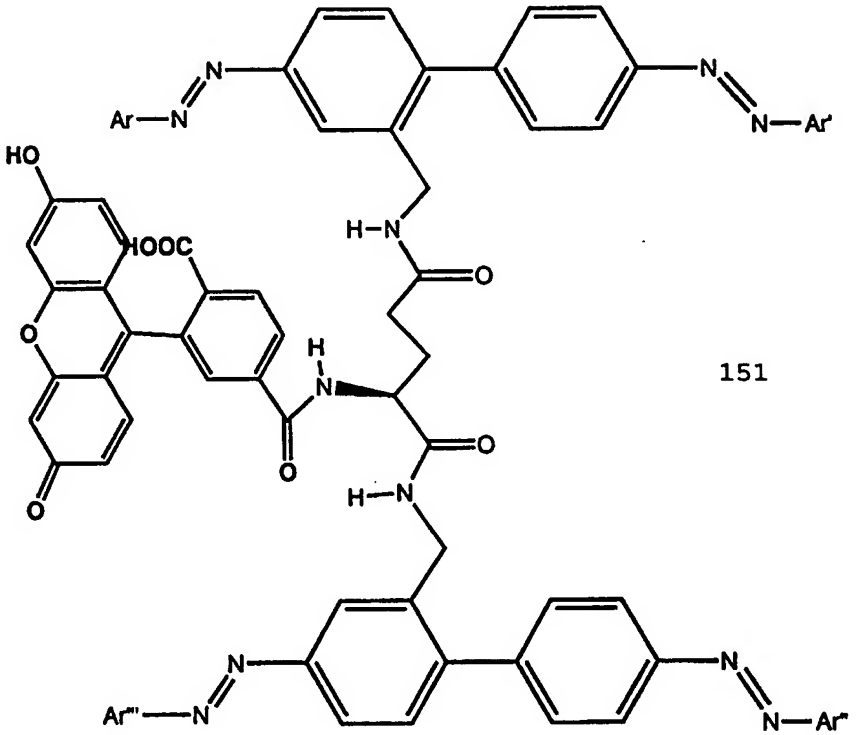
L and L' are -N=N-, -CONH-, -NHCO-, -HN-NH-, or -C=C-, and can be the same or different from each other; and

Ar and Ar' are



and can be the same or different from each other, where each of V, W, Q, Y, Z, A and B is OH, COOH, H, R^5 (R^5 is C_{1-6} hydrocarbon), CO_2R^6 (R^6 is C_{1-6} hydrocarbon), $CONH_2$, CN, NH_2, CH_2NH_2 or SO_3 and can be the same or different from each other.

A preferred compound of the fluorescent dimer of formula IX is



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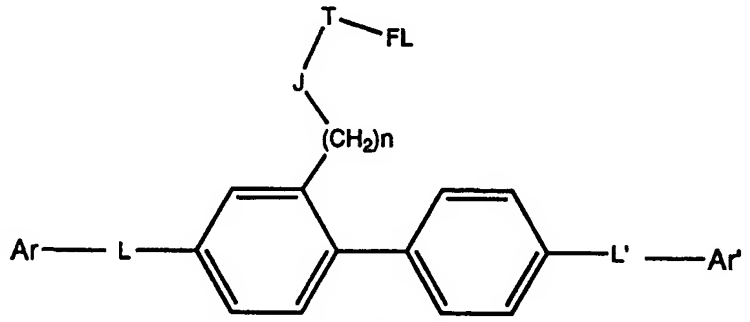
The fluorescent dimeric compounds of formula IX can be used, e.g., to measure aggregated forms of β-amyloid protein in cultured cells, e.g., in an in vitro assay for screening for compounds which inhibit aggregation in a physiologically-relevant location. An advantage of these dimeric compounds is that they have increased affinity for binding amyloid as compared to the related monomeric compounds.

The invention also provides and amyloid binding compound of the formula



(X)

and pharmaceutically acceptable salts thereof,

wherein

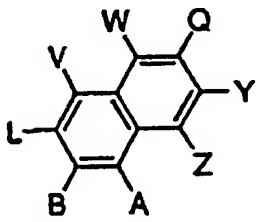
J is NH or S;

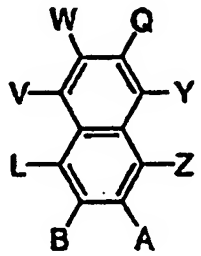
T is CO or CO_2;

FL is fluorescein, rhodamine, coumarin, or any other fluorescent moiety;

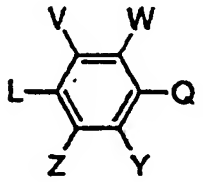
L and L' are -N=N-, -CONH-, -NHCO-, -HN-NH-, or -C=C-, and can be the same or different from each other; and

Ar and Ar' are





or



and can be the same or different from each other, where each of V, W, Q, Y, Z, A and B is OH, COOH, H, R^5 (R^5 is C_{1-6} hydrocarbon), CO_2R^6 (R^6 is C_{1-6} hydrocarbon), $CONH_2$, CN, NH_2, CH_2NH_2 or SO_3 and can be the same or different from each other.

The fluorescent monomeric compounds of formula X can be used, e.g., to measure aggregated forms of β-amyloid protein in cultured cells, e.g., in an in vitro assay for screening for compounds which inhibit aggregation in a physiologically-relevant location.

This invention also provides a method for diagnosing the degree of progression of Alzheimer's disease in a mammal. A first mammal having Alzheimer's disease and having brain amyloid fibrils is provided. A labeled ligand capable of interacting with the amyloid fibrils is also provided. The labeled ligand is administered to the mammal under conditions which allow the labeled ligand to interact with the amyloid fibrils in the brain so as to result in labeled amyloid fibrils. The localization or quantification of the labeled amyloid fibrils in the mammal is determined by imaging so as to diagnose the degree of progression of the Alzheimer's disease.

Mammal is meant to include human and non-human mammals. Alzheimer's disease is meant to include human Alzheimer's disease and similar diseases in other mammals characterized by Alzheimer's disease-like amyloidosis. Alzheimer's disease is a progressively deteriorating disease which eventually leads to death. A mammal having Alzheimer's disease is meant to include stages of the disease in which the symptoms of the disease are apparent or not apparent.

By amyloid fibril is meant aggregated amyloid protein. Amyloid fibril is meant to include any aggregated precursor, intermediate or mature form of amyloid protein that is formed in vitro or in vivo. Examples of amyloid proteins include β-amyloid, NAC, Islet

amyloid polypeptide (IAPP), immunoglobulin G (Ig) light chain, transthyretin, lysozyme, β_2-microglobulin and prion protein. β-amyloid proteins are meant to include Aβ1-42 and Aβ1-40 (a C-terminally truncated relative of Aβ1-42), Aβ17-42, Aβ3-42 (pyro Glu), as well as other length β-amyloid peptides. Under certain conditions, amyloid proteins aggregate. Examples of aggregated β-amyloid, i.e., β-amyloid fibrils, include protofibrils, type-1 fibrils, type-2 fibrils, neuritic plaque, diffuse amyloid, and combinations thereof.

Protofibrils are a distinct oligomeric aggregate of β-amyloid which form during the early phases of in vitro fibril formation by synthetic β-amyloid peptides (e.g., Aβ1-40 and Aβ1-42). Using atomic force microscopy (AFM), protofibrils appear as small elongated β-amyloid oligomers which become detectable during the early phase of aggregation and elongate slowly with time before rapidly disappearing following the appearance of prototypical amyloid fibrils. Average protofibril heights measured by AFM are 3.1 nm + 0.31 nm for Aβ1-40 and 4.2 + 0.58 nm for Aβ1-42. Protofibril lengths are similar for both Aβ1-40 and Aβ1-42 and are as short as 20 nm at early time points and gradually increase with longer incubation times to reach lengths commonly exceeding 200 nm. These protofibrils are further characterized by small periodic increases in diameter with a periodicity of 20-22 nm which are observed by AFM for both Aβ1-40 and Aβ1-42. The protofibril is the precursor to the type-1, type-2 and neuritic β-amyloid fibrils. In a preferred embodiment, it is the protofibrils in the mammal that interact with the labeled ligand.

Type-1 and type-2 fibrils are formed during in vitro synthesis of β-amyloid peptides and are examples of prototypical β-amyloid fibrils. Type-1 and type-2 fibrils have lengths rarely less than about 1 μm and often greater than about 5μm, as observed using AFM. Type-1 fibrils have larger apparent diameters than protofibrils (7.8 + 0.45 nm for Aβ1-40 and 7.3 + 0.53 nm for Aβ1-42), and a left-handed helical twist that creates periodic increases in diameter along the long axis of the fibril with a period of approximately 43 nm (for both Aβ1-40 and Aβ1-42). Type-1 fibrils may be composed of protofibrils wrapped around each other. Type-2 fibrils have average heights measured by AFM of about 4-6 nm. Type-2 lack the period increases in diameter which characterize both protofibrils and type-1 fibrils. Instead, they display discontinuities at less regular intervals, often in excess of 100 nm, which make the fibrils appear to be composed of smooth segments assembled linearly with the ends slightly offset.

Neuritic plaques contain aggregated β-amyloid fibrils, e.g., type-1 and type-2 fibrils and protofibrils. Neuritic plaques have classically been defined as the disease-associated

fibrils in, e.g., Alzheimer's disease. Postmortem brains of persons having Alzheimer's disease are characterized by the presence of such β-amyloid neuritic plaques. In Alzheimer's disease, the predominant brain amyloid proteins are Aβ1-42 and its C-terminally truncated relative Aβ1-40.

By diffuse amyloid is meant Aβ1-40 and Aβ1-42 aggregates that appear amorphous using electron microscopy. Diffuse amyloid characterizes the brains of individuals who are predisposed to Alzheimer's disease. Diffuse amyloid does not contain type-1 or type-2 fibrils, but may contain protofibrils which cannot be detected by electron microscopic examination of diseased tissue. Detection of protofibrils thus can provide a method for detecting aggregated β-amyloid earlier than is possible by targeting later-stage fibrils, and therefore can be used as a diagnostic tool for identifying individuals predisposed to Alzheimer's disease. Stabilization of the protofibril intermediate can also inhibit formation of later-stage fibrils.

The ligand of this invention is capable of interacting with amyloid fibrils. In certain embodiments, the ligand is capable of interacting specifically with brain amyloid fibrils. In certain embodiments, the ligand is capable of interacting specifically with only certain types of amyloid fibrils, e.g., it interacts with β-amyloid protofibrils, but not with β-amyloid fibrils, or it interacts with type-1 or type-2 β-amyloid fibrils but not with diffuse amyloid. Interacts is meant to include, e.g., binds, complexes, associates, or conjugates. In certain embodiments, the ligand is, e.g., an aromatic azo dye, e.g., an analog of Congo Red or Chrysamine G. Preferably, the ligand is an organometallic ligand. By organometallic ligand is meant a ligand which has organic parts and metallic parts.

Any label whose presence in the mammal can be determined by non-invasive procedures can be used. For example, the label in the labeled ligand can be a gamma emitter. Preferred gamma emitters are technetium-99m, indium-111, yttrium-90 and rhenium-186. Technetium-99m is most preferred. A preferred beta emitter is technetium-99. Preferably, the labeled ligand is an organometallic ligand.

In a preferred embodiment, the labeled ligand is a compound of formula **I**, or a pharmceutically acceptable salt thereof described above. Preferred compounds of formula **I** are compounds of formulas **3, 6, 15, 28, 35, 45, 54** and **66**, or pharmaceutically acceptable salts thereof, described above. Most preferred compounds of formula **I** are formulas **3** or **6**, or a pharmaceutically acceptable salt thereof.

Other preferred compounds are compounds of formulas **II** or **III** or pharmaceutically

acceptable salts thereof, described above. Examples of compounds of formula II are **140-143**, and examples of compounds of formula III are **144-147**, described above. Most preferably, the compounds are the dimers of formula IV or V, or pharmaceutically acceptable salts thereof, described above.

Dimers are preferred because Congo Red does not specifically stain amyloid in Alzheimer's disease brain tissue. Rather, it is concentrated in plaque and appears as a green stain when a tissue section is viewed under polarized light. This phenomenon, known as birefringence, is specific to amyloid and arises because the Congo Red molecules are aligned in an ordered array along the fibril surface. It is believed that other interactions of Congo Red in tissue involve 1:1 interactions. The specificity of ordered multivalent binding is exploited by the covalent dimers of the compounds of this invention. Without being bound by any theory, these dimers should exhibit increased affinity for the fibril surface, but not have increased affinity for nonspecific, i.e., 1:1, binding partners. The increased affinity results from the fact that the covalent dimer loses less entropy on binding (i.e., the entropy of binding will be less unfavorable) than do two separate molecules. Accordingly, covalent dimers in which the two Congo Red-based subunits are rigidly held in the geometry required for fibril binding, should lose no entropy on binding and therefore have an even greater affinity. The rigidification of the dimer "linker" can be accomplished by the combinatorial approach discussed infra. The entropic advantage enjoyed by the dimer is even greater for trimers or higher oligomers. Trimers and higher oligomers are also meant to be covered by this invention. Trimers can be prepared using similar chemistry as for the synthesis of the dimers.

In certain embodiments, the labeled ligand is capable of interacting specifically with brain amyloid fibrils. In preferred embodiments, the labeled ligand is capable of interacting specifically with one kind of amyloid fibril, e.g., type-1 fibrils or type-2 fibrils. Most preferably, the labeled ligand is capable of interacting specifically with β-amyloid protofibrils.

Administration of the labeled ligand into the mammal can be accomplished by any method which allows the labeled ligand to interact with amyloid fibrils in the brain so as to result in labeled amyloid fibrils. These methods include, e.g., injection, infusion, deposition, implantation, oral ingestion or topical administration, or any other method of administration where access to the brain by the labeled ligand is obtained. Preferably, administration is by injection. Injections can be, e.g., intravenous, intradermal,

subcutaneous, intramuscular or intraperitoneal. Single or multiple administrations of the ligand can be given. In certain embodiments, a combination of different ligands are given. In other embodiments, the labeled ligand can be administered with one or more additional materials.

The labeled ligand can be in a liquid, e.g., in dissolved form or colloidal form. The liquid can be a solvent, partial solvent or non-solvent. In many cases, water or an organic liquid can be used.

The dose of the labeled ligand that is administered is determined in part by the amount of radioactivity that is desirable to administer. The amount of radioactivity depends upon the isotope used and can be determined by one skilled in the art without undue experimentation. For example, it is preferred to use about 0.1 mCi/dose to about 100 mCi/dose.

The labeled ligand needs to cross the blood brain barrier so as to reach the brain. By blood brain barrier is meant capillary endothelial cells with continuous tight junctions and with no detectable transendothelial pathways. Such structures provide a cellular barrier between the blood and the interstitial fluid, thus controlling the exchange of materials between the blood and the central nervous system. In certain embodiments, the labeled ligand itself is able to cross the blood brain barrier. See, e.g., Tubis et al., J. Am. Pharmaceutical Assoc. 49:422-425 (1960), which reports that a tetra-iodinated form of Congo Red crosses the blood brain barrier to the extent of 0.03% of a peritoneally injected dose. Such an amount results in nM levels of the compound in the brain, which is sufficient for SPECT imaging. It is preferred to use electrically neutral ligands to cross the blood brain barrier. Examples of electrically neutral compounds of this invention are compounds of formulas **II**, **III** and **IV**, and some versions of **V**, **VII** and **VIII**. In other embodiments, materials are administered so as to aid the labeled ligand to cross the blood brain barrier. For example, mannitol, an organic solvent, certain drugs, or RMP-7 can be used to open the blood brain barrier so as to allow the labeled ligand to enter. Any method for opening the blood brain barrier known to those skilled in the art can be used in this invention.

The labeled ligand interacts with the amyloid fibrils in, e.g., the brain, so as to result in labeled amyloid fibrils. The localization or quantification of the labeled amyloid fibrils is determined by imaging. By imaging is meant the detection of the distribution of the label in the body by non-invasive means. Examples of imaging include radioimaging, magnetic resonance imaging and single photon emission computed tomographic imaging. The

presence of gamma emitters can be determined, e.g., by a gamma camera or a single photon emission computed tomography (SPECT) camera. The presence of positron emitters can be determined, e.g., by a positron emission tomographic (PET) camera. These imaging techniques are known to those skilled in the art. The imaging can be a total body scan or a partial body scan, e.g., of the brain. The timing after administration of the labeled ligand for a scan can be minutes, hours, days or weeks.

Depending upon the localization and/or quantification of the labeled amyloid fibrils, a diagnosis is made regarding the degree of progression of the Alzheimer's disease. In certain embodiments, the localization or quantification of the labeled amyloid fibrils is compared to a standard. The standard can be, e.g., the localization or quantification pattern of a second mammal not having Alzheimer's disease, or the localization or quantification pattern obtained from an earlier determination of the first mammal.

The invention also provides a method for monitoring the response to a therapy in a mammal having Alzheimer's disease. A mammal having Alzheimer's disease and having brain amyloid fibrils is provided. The mammal is treated with a therapy for Alzheimer's disease. The response of the mammal to the treating step is monitored by determining whether the therapy alters the localization or quantification of the amyloid fibrils in the mammals.

Therapy is meant to include, e.g., compounds, mixtures of compounds, radiation, ultrasound, or any other type of treatment which can alleviate Alzheimer's disease.

In certain embodiments, the labeled ligand is capable of interacting specifically with brain amyloid fibrils. In preferred embodiments, the labeled ligand is capable of interacting specifically with one kind of amyloid fibril, e.g., type-1 fibrils or type-2 fibrils. Most preferably, the labeled ligand is capable of interacting specifically with β-amyloid protofibrils.

In preferred embodiments, the determining step comprises providing a labeled ligand capable of interacting with the amyloid fibrils. The labeled ligand is administered to the mammal under conditions which allow the labeled ligand to interact with the amyloid fibrils in the brain so as to result in labeled amyloid fibrils. The localization or quantification of the labeled amyloid fibrils in the mammal is determined by imaging. Preferably, the labeled ligand is a compound of formula I, or a pharmaceutically acceptable salt thereof, described above. Preferred compounds of formula I are compounds of formulas **3, 6, 15, 28, 35, 45, 54** or **66**, or a pharmaceutically acceptable salt thereof, described above. Other preferred

compounds are compounds of formulas II or III or pharmaceutically acceptable salts thereof, described above. Examples of compounds of formula II are 140-143, and examples of compounds of formula III are 144-147, described above. Most preferably, the compounds are the dimers of formula IV or V, or pharmaceutically acceptable salts thereof, described above.

The invention also provides a method for evaluating the ability of an agent to alter the localization or quantification of brain amyloid fibrils in a mammal. A mammal having brain amyloid fibrils is provided. An agent is provided. The agent is administered to the mammal and it is determined whether the agent alters the localization or quantification of the brain amyloid fibrils in the mammal.

Altering is meant to include directly or indirectly altering the localization or quantification of brain amyloid fibrils. For example, an agent can affect some condition or factor which in turn affects the localization or quantification of brain amyloid fibrils, or the agent can directly affect the localization or quantification.

The agent being tested can be, e.g., a putative therapeutic agent for a disease, e.g., Alzheimer's disease, or it can be a putative agent which alters other abnormal states which affect localization or quantification of brain amyloid fibrils, or it can be a putative agent which alters the normal localization or quantification of brain amyloid fibrils. Agents can include, e.g., proteins, peptides, carbohydrates, polysaccharides, glycoproteins, nucleic acids, peptidomimetics, organic molecules (preferably, less than 1500 kDa), fragments or recombinant forms of the above, or any other type of compound which can be administered to the mammal. Agents are also meant to include, e.g., ionizing radiation, non-ionizing radiation and ultrasound. Agents include, e.g., inhibitors or activators of a molecule that is either required for, or inhibits, the synthesis, post-translational modification or functioning of some element involved in the localization or quantification of amyloid. Agents can, e.g., regulate the spatial or temporal control of expression of a gene product. Agents can include, e.g., cytokines, growth factors, hormones, signaling components, kinases, phosphatases, homeobox proteins, transcription factors, translation factors and post-translational factors or enzymes. Agents can, e.g., make modifications, e.g., chemical, charge or shape modifications, in the amyloid or some other element. Agents can, e.g., affect the interaction between two or more cellular or extra-cellular components. The agent can, e.g., act directly or indirectly on the amyloid so as to alter the localization or quantification of the amyloid. The agent can be specific or non-specific for affecting amyloid localization or quantification.

The agents of the invention are meant to include reversible and non-reversible agents.

Administration of the agent can be accomplished by any method which allows the agent to reach its target. These methods include, e.g., injection, infusion, deposition, implantation, suppositories, oral ingestion, inhalation or topical administration, or any other method of administration where access to the target by the agent is obtained. Injections can be, e.g., intravenous, intradermal, subcutaneous, intramuscular or intraperitoneal. Implantation includes inserting implantable drug delivery systems, e.g, microspheres, hydrogels, polymeric reservoirs, cholesterol matrices, polymeric systems, e.g., matrix erosion and/or diffusion systems and non-polymeric systems, e.g., compressed, fused or partially fused pellets. Suppositories include glycerin suppositories. Oral ingestion doses can be enterically coated. Inhalation includes administering the agent with an aerosol in an inhalator, either alone or attached to a carrier that can be absorbed.

The agent can be suspended in a liquid, e.g, in dissolved form or colloidal form. The liquid can be a solvent, partial solvent or non-solvent. In many cases water or an organic liquid can be used.

Administration of the agent can be alone or in combination with other therapeutic agents or other materials. In certain embodiments, materials are administered so as to aid the agent in crossing the blood brain barrier. In certain embodiments, the agent can be combined with a suitable carrier, e.g., a pharmaceutically acceptable carrier, incorporated into a liposome, or incorporated into a polymer release system.

In certain embodiments of the invention, the administration can be designed so as to result in sequential exposures to the agent over some time period, e.g., hours, days, weeks, months or years. This can be accomplished by repeated administrations of the agent by one of the methods described above, or alternatively, by a controlled release delivery system in which the agent is delivered to the mammal over a prolonged period without repeated administrations. By a controlled release delivery system is meant that total release of the agent does not occur immediately upon administration, but rather is delayed for some time period. Release can occur in bursts or it can occur gradually and continuously. Administration of such a system can be, e.g., by long acting oral dosage forms, bolus injections, transdermal patches and sub-cutaneous implants.

Examples of systems in which release occurs in bursts include, e.g, systems in which the agent is entrapped in liposomes which are encapsulated in a polymer matrix, the liposomes being sensitive to a specific stimuli, e.g., temperature, pH, light or a degrading

enzyme, and systems in which the agent is encapsulated by an ionically-coated microcapsule with a microcapsule core-degrading enzyme. Examples of systems in which release of the agent is gradual and continuous include, e.g., erosional systems in which the agent is contained in a form within a matrix, and diffusional systems in which the agent permeates at a controlled rate, e.g., through a polymer. Such sustained release systems can be, e.g., in the form of pellets or capsules.

In preferred embodiments, the localization and quantification is determined by the methods described above. The localization or quantification is altered if, e.g., it differs from a standard localization or quantification pattern. The standard used can be, e.g., the pattern obtained from the same mammal when the agent is not present in the mammal, or the pattern obtained from another mammal.

Preferably, the labeled ligand is a compound of formula I or a pharmaceutically acceptable salt thereof, described above. Preferred compounds of formula I are compounds of formulas **3, 6, 15, 28, 35, 45, 54** or **66**, or a pharmaceutically acceptable salt thereof, described above. Other preferred compounds are compounds of formulas II or III or pharmaceutically acceptable salts thereof, described above. Examples of compounds of formula II are **140-143**, and examples of compounds of formula III are **144-147**, described above. Most preferably, the compounds are the dimers of formula IV or V, or pharmaceutically acceptable salts thereof, described above.

In certain embodiments, the labeled ligand is capable of interacting specifically with brain amyloid fibrils. In preferred embodiments, the labeled ligand is capable of interacting specifically with one kind of amyloid fibril, e.g., type-1 fibrils or type-2 fibrils. Most preferably, the labeled ligand is capable of interacting specifically with β-amyloid protofibrils.

The invention also includes a method for identifying an agent useful for treating a mammal having a disease associated wtih aggregated amyloid. A mammal having such a disease and having amyloid fibrils is provided. An agent is provided and administered to the mammal. It is determined if the agent alters the localization or quantification of the amyloid fibrils in the mammal. An alteration in the localization or quantification which results in a localization or quantification more similar to that of a mammal which does not have the disease is correlated with the agent being useful for treating the mammal having the disease. Treating is meant to include, e.g., preventing, treating, reducing the symptoms of, or curing the disease.

The disease can be any disease which is associated with aggregated amyloid, e.g., Alzheimer's disease (aggregated β-amyloid), type II diabetes (aggregated Islet amyloid polypeptide), B-cell lymphoma (aggregated Ig light chain), Creutzfeldt-Jacob disease or bovine spongiform encephelopathy (aggregated prion protein), familial transthyretin amyloidosis (aggregated transthyretin), complications from dialysis (aggregated β_2-microglobulin), or other systemic amyloidoses (e.g., aggregated lysozyme), e.g., serum amyloid A systemic amyloidosis.

In preferred embodiments, the disease is Alzheimer's disease. In such embodiments, it is preferrerd that the labeled ligand is capable of interacting specifically with brain amyloid fibrils, preferably with one kind of amyloid fibril, e.g., type-1 fibrils or type-2 fibrils, and most preferably with β-amyloid protofibrils.

In preferred embodiments, the localization and quantification is determined by the methods described above. Preferably, the labeled ligand is a compound of formula I or a pharmaceutically acceptable salt thereof, described above. Preferred compounds of formula I are compounds of formulas **3, 6, 15, 28, 45, 54** or **66**, or a pharmaceutically acceptable salt thereof, described above. Other preferred compounds are compounds of formulas **II** or **III** or pharmaceutically acceptable salts thereof, described above. Examples of compounds of formula **II** are **140-143**, and examples of compounds of formula **III** are **144-147**, described above. Most preferably, the compounds are the dimers of formula **IV** or **V**, or pharmaceutically acceptable salts thereof, described above. Administration of the agent is as described above.

The invention also includes the agent obtainable by this method.

The invention further includes a method for determining the localization or quantification of amyloid fibrils in a mammal. A mammal having amyloid fibrils is provided. An organometallic ligand capable of interacting with the amyloid fibrils is provided. The organometallic ligand is administered to the mammal under conditions which allow the organometallic ligand to interact with the amyloid fibrils so as to result in organometallic ligand-amyloid fibril complexes. The localization or quantification of the complexes is determined in the mammal.

The amyloid fibrils can be anywhere in the body of the mammal. For example, the amyloid fibrils can be in the brain, pancreas, vasculature, spleen, liver, kidneys, adrenals, lymph nodes, muscle, cardiovascular system, skin, or any combination thereof. The organometallic ligands of this invention are useful, e.g., for detecting amyloid plaques

characteristic of degenerating tissue, e.g., from Alzheimer's disease in the brain, type II diabetes in the pancreas, B-cell lymphoma in the vasculature, Creutzfeldt-Jacob disease in the brain, familial transthyretin amyloidosis in the liver and other peripheral sites, or complications from dialysis in the kidneys.

The organometallic ligands of this invention can have different relative affinities for the different types of amyloid fibrils. Thus, in addition to the general affinity of the organometallic ligands for all amyloid fibrils, there is a specific component to the affinity which depends on precise interactions between the organometallic ligand and the amyloid fibril. Therefore, certain of the organometallic ligands of this invention can be used to distinguish amyloid fibrils of different composition, e.g., the brain amyloid proteins β1-40 and NAC. NAC is a minor brain amyloid protein. NAC may represent up to 10% by moles of Alzheimer's disease brain amyloid fibrils. NAC fibrils seed polymerization of β1-40 *in vitro* and *vice versa*. Han et al., Chem. Biol. 2:163-169 (1995).

In certain embodiments, the labeled ligand is capable of interacting specifically with brain amyloid fibrils. In preferred embodiments, the labeled ligand is capable of interacting specifically with one kind of amyloid fibril, e.g., type-1 fibrils or type-2 fibrils. Most preferably, the labeled ligand is capable of interacting specifically with β-amyloid protofibrils.

In preferred embodiments, the localization and quantification is determined by the methods described above. Preferably, the organometallic ligand is a compound of formula I or a pharmaceutically acceptable salt thereof, described above. Preferred compounds of formula I are compounds of formulas **3, 6, 15, 28, 45, 54** or **66**, or a pharmaceutically acceptable salt thereof, described above. Other preferred compounds are compounds of formulas II or III or pharmaceutically acceptable salts thereof, described above. Examples of compounds of formula II are **140-143**, and examples of compounds of formula III are **144-147**, described above. Most preferably, the compounds are the dimers of formula IV or V, or pharmaceutically acceptable salts thereof, described above. The complexes formed between the organometallic ligand and the amyloid fibril can result from, e.g., bonding, associating, complexing or conjugating. It is preferred that the determining step is by imaging.

In certain embodiments, the administering and determining steps are repeated after a time interval so as to establish a time course for the localization or quantification of the complexes in the mammal. Preferably the time intervals are about 1 minute to about 24

hours, most preferably they are about 30 minutes to about 6 hours. These steps can be repeated one or more times.

In certain embodiments, the mammal is deceased, and the administering step is, e.g., to the postmortem brain or a portion thereof. In such embodiments, the determining step can be, e.g., by autoradiography, SPECT, PET or magnetic resonance imaging.

The invention also includes a method for treating Alzheimer's disease in a mammal. A mammal having Alzheimer's disease is provided. The mammal has non-aggregated amyloid proteins or aggregated amyloid proteins, or combinations thereof. An organometallic ligand capable of interacting with the non-aggregated amyloid proteins, or with the aggregated amyloid proteins, or with both of the amyloid proteins, is provided. A therapeutically effective amount of the organometallic ligand is administered to the mammal under conditions which allow the organometallic ligand to interact with the non-aggregated amyloid proteins, or with the aggregated amyloid proteins, or with both of the amyloid proteins, so as to inhibit aggregation of the amyloid proteins such that treatment of the Alzheimer's disease occurs.

Aggregated amyloid proteins is meant to include fully or partially aggregated amyloid proteins. By partially aggregated amyloid proteins is meant that aggregation of additional amyloid proteins onto the existing aggregated amyloid proteins, e.g., protofibrils or fibrils, can occur under the appropriate conditions. In certain embodiments, the aggregated amyloid proteins are Alzheimer's disease associated β-amyloid fibrils. In certain embodiments, the aggregated amyloid proteins are protofibrils, type-1 fibrils, type-2 fibrils, neuritic plaque, diffuse amyloid, or combinations thereof.

By inhibit aggregation is meant, e.g., that the amyloid proteins are unable to properly interact with each other to effect, e.g., formation of, or growth of, aggregates of amyloid proteins, e.g, the growth of protofibrils, the conversion of protofibrils into fibrils, the growth of fibrils and the growth of neuritic plaque. Inhibiting interaction is also meant to include reversing aggregation of the amyloid proteins.

In a preferred embodiment, the organometallic ligand specifically interacts with β-amyloid protofibrils. Stabilization or inactivation of the protofibril inhibits formation of later-stage fibrils.

Preferably, the organometallic ligand is a compound of formula I or a pharmaceutically acceptable salt thereof, described above. Other preferred compounds are compounds of formulas II and III describe above. Most preferred compounds are the dimers

of formula IV or V described above. It is preferred that a non-radioactive organometallic ligand is used for treatment, so as to avoid potential toxicity from high dosages of radioactive organometallic ligands. Examples of non-radioactive metals that can be used in compounds of formula I and VI include Cd, Zn, Co, Cu, Fe, Ni, or combinations thereof. Oxo forms of these metals can also be used. The concentration of the organometallic ligand that is administered is at a dose about 1 to about 1500 mg/kg body weight. Preferably, the dose is about 2 to about 200 mg/kg body weight. Most preferably, the dose is about 2 to about 20 mg/kg body weight. Preferably, the dosage form is such that it does not substantially deleteriously affect the mammal.

Administration of the organometallic ligand is as described above. By therapeutically effective amount is meant that amount which is capable of at least partially preventing or reversing aggregation of the amyloid proteins. A therapeutically effective amount can be determined on an individual basis and will be based, at least in part, on consideration of the species of mammal, the mammal's size, the organometallic ligand used, the type of delivery system used, the time of administration relative to amyloid protein aggregation formation, and whether a single, multiple, or controlled release dose regimen is employed. A therapeutically effective amount can be determined by one of ordinary skill in the art employing such factors and using no more than routine experimentation.

In certain embodiments, the treatment can be assessed by determining the localization and/or quantification of any remaining amyloid fibrils after treatment, by the methods described above. Treating is meant to include, e.g., preventing, treating, reducing the symptoms of, or curing the disease.

The invention also includes a method for treating Alzheimer's disease in a mammal that is similar to the method described above, except that the ligands used are compounds of formulas II, III, IV and V, or pharmaceutically acceptable salts thereof, without any metals bound to them.

The invention also includes a pharmaceutical composition for treating Alzheimer's disease in a mammal comprising a therapeutically effective amount of an organometallic ligand, the ligand being able to interact with amyloid proteins in a mammal in need of treatment for Alzheimer's disease, and a pharmaceutically acceptable carrier. Preferably, the organometallic ligand is a compound of formula I or a pharmaceutically acceptable salt thereof, described above. More preferred compounds are compounds of formulas II or III described above. Most preferably, the compounds are the dimers of formula IV or V

described above. Pharmaceutical compositions for treating other diseases associated with aggregated amyloid are also included in this invention. Preferably, non-radioactive organometallic ligands are used.

The invention also includes pharmaceutical compositions that are similar to the pharmaceutical compositions described above, except that the compounds used are compounds of formulas **II**, **III**, **IV** and **V**, and pharmaceucially acceptable salts thereof, without any metals bound to them.

In certain embodiments, the labeled ligand is capable of interacting specifically with brain amyloid fibrils. In preferred embodiments, the labeled ligand is capable of interacting specifically with one kind of amyloid fibril, e.g., type-1 fibrils or type-2 fibrils. Most preferably, the labeled ligand is capable of interacting specifically with β-amyloid protofibrils.

The invention also includes a method for determining the localization or quantification of amyloid fibrils in a deceased mammal. A deceased mammal or a portion thereof having amyloid fibrils is provided. An organometallic ligand capable of interacting with the amyloid fibrils is provided. The organometallic ligand is administered to the mammal or portion thereof under conditions which allow the organometallic ligand to interact with the amyloid fibrils so as to result in organometallic ligand-amyloid fibril complexes. The localization or quantification of the complexes in the mammal or portion thereof is determined, e.g., by autoradiography, SPECT, PET or magnetic resonance imaging.

Preferably, the organometallic ligand is a compound of formula **I** or a pharmaceutically acceptable salt thereof, described above. Preferred compounds of formula **I** are compounds of formulas **3**, **6**, **15**, **28**, **35**, **45**, **54** or **66**, or a pharmaceutically acceptable salt thereof, described above. Other preferred compounds are compounds of formulas **II** or **III** or pharmaceutically acceptable salts thereof, described above. Examples of compounds of formula **II** are **140-143**, and examples of compounds of formula **III** are **144-147**, described above. Most preferably, the compounds are the dimers of formula **IV** or **V**, or pharmaceutically acceptable salts thereof, described above.

The invention also includes a method for detecting the presence of aggregated prion protein in a mammal. A mammal is provided. Bodily fluid or tissue obtained from the mammal is provided. A labeled ligand capable of interacting with aggregated prion protein is provided. The bodily fluid or tissue is contacted *in vitro* with the labeled ligand under conditions which allow the ligand to interact with the aggregated prion protein if the

aggregated prion protein is present in the bodily fluid or tissue, so as to result in labeled aggregated prion protein. The presence or absence of the labeled aggregated prion protein in the bodily fluid or tissue is determined.

By prion protein is meant the infectious agent of a prion disease. The prion diseases are a group of transmissible neurodegenerative diseases which infect mammals, e.g., cows, sheep, and humans. In humans, the disease, known as Creutzfeldt-Jacob disease, resembles Alzheimer's disease with respect to symptoms and neuropathology. The majority of cases of prion disease are in the livestock population, e.g., in sheep (scrapie) and cows (bovine spongiform encephalopathy, BSE, also known as "mad cow disease"). The human prion disease is extremely rare and usually strikes elderly patients. It has been reported that BSE can be transmitted into humans via infected beef. Currently, there is no therapeutic agent for the prion diseases. There is a need for a sensitive, simple, and practical method to detect the infectious agent. For example, such a detection method would allow infected livestock, e.g., cows, to be selectively destroyed.

The infectious agent, or prion, is an aggregated form of a normal protein. Although the sequence of the prion protein differs from the amyloid protein of Alzheimer's disease, there is a significant similarity which may account for their similar structure: the prion often exists as an amyloid fibril. The ligands of this invention have affinity for prion.

Bodily fluid is meant to include any fluid from the mammal, e.g., lymph, blood, or urine. Preferably, the bodily fluid used is lymph. In certain preferred embodiments, the bodily fluid is filtered *in vitro* such that any prion infectious agent that is present in the bodily fluid does not pass through the filter. For example, a Millipore Ultrafree-MC polysulfone membrane, 300,000 NMWL cutoff, can be used. The filter is then contacted with the labeled ligand. Preferably, the presence of resulting labeled prion protein is determined using a SPECT detector, though any other method known to those skilled in the art to detect the labeled prion can also be used.

Preferably, the labeled ligand is a compound of formula I or a pharmaceutically acceptable salt thereof, described above. Preferred labels are the gamma or positron or beta emitters described above, however, any label known to those skilled in the art which is capable of being detected *in vitro* can be used. Preferably, the labeled ligand of formula I is a compound of formulas **3, 6, 15, 28, 35, 45, 54** or **66**, or a pharmaceutically acceptable salt thereof, described above. Other preferred compounds are compounds of formulas **II** or **III** or pharmaceutically acceptable salts thereof, described above. Examples of compounds

of formula II are **140-143**, and examples of compounds of formula III are **144-147**, described above. Most preferably, the compounds are the dimers of formula IV or V, or pharmaceutically acceptable salts thereof, described above.

Advantages of using the labeled ligands of this invention include that they are very sensitive, and are much cheaper to make and more chemically stable than antibodies. In addition, antibody tests do not distinguish the infectious form of the protein, the prion, which is usually fibrillar, from the normal, innocuous form of the protein.

The invention also includes another method for detecting the presence of aggregated prion protein in a mammal. A mammal is provided. A labeled ligand capable of interacting with aggregated prion protein is provided. Preferably, the labeled ligand is a compound of formula I or a pharmaceutically acceptable salt thereof, described above. Preferred compounds of formula I are compounds of formulas **3, 6, 15, 28, 35, 45, 54** or **66**, or a pharmaceutically acceptable salt thereof, described above. Other preferred compounds are compounds of formulas II or III or pharmaceutically acceptable salts thereof, described above. Examples of compounds of formula II are **140-143**, and examples of compounds of formula III are **144-147**, described above. Most preferably, the compounds are the dimers of formula IV or V, or pharmaceutically acceptable salts thereof, described above. The labeled ligand is administered to the mammal under conditions which allow the labeled ligand to interact with the aggregated prion protein if the aggregated prion protein is present in the mammal, so as to result in labeled aggregated prion protein. The presence or absence of labeled aggregated prion protein is determined in the mammal by imaging.

The invention also includes using the labeled ligands to prevent aggregated prion formation in cell culture.

The invention also includes a method for determining the presence of aggregated intracellular β-amyloid. Cells having β-amyloid are provided. A fluorescent ligand capable of interacting with aggregated β-amyloid is provided. The cells are contacted with the fluorescent ligand under conditions which allow the fluorescent ligand to interact with aggregated β-amyloid if it is present so as to result in fluorescent-labeled aggregated β-amyloid. The presence or absence of a fluorescent signal is determined. The presence of a fluorescent signal indicates the presence of aggregated intracellular β-amyloid.

In certain embodiments, the cells are permeabilized prior to contacting the cells with the fluorescent ligand. Preferably, the fluorescent ligand is a compound of formula IX or X, or pharmaceutically acceptable salts thereof. Examples include Congo Red fluorescein

ligand **131**, Congo Red rhodamine ligand **133** and Congo Red coumarin ligand **135**. Other fluorescent labels known to those skilled in the art can also be used. The fluorescent ligands are particularly useful as cell culture probes.

The invention also includes a method for identifying an agent useful for treating a mammal for a disease characterized by aggregated intracellular β-amyloid. Cells having β-amyloid are provided. An agent is provided. A fluorescent ligand capable of interacting with β-amyloid fibrils is provided. The cells are contacted with the agent to form a mixture under conditions which allow aggregation of the β-amyloid if the agent was not present. The mixture is contacted with the fluorescent ligand under conditions which allow the fluorescent ligand to interact with β-amyloid fibrils if they are present so as to result in fluorescent-labeled β-amyloid fibrils. It is determined if the agent inhibits aggregation of the β-amyloid. The presence of a fluorescent signal indicates the presence of β-amyloid fibrils and therefore minimal or no inhibition by the agent. The absence of a fluorescent signal indicates the absence of β-amyloid fibrils and therefore inhibition by the agent. This inhibition is correlated with the agent being useful for treating a mammal for a disease characterized by aggregated intracellular β-amyloid.

This method can be used for any disease which is characterized by aggregated intracellular β-amyloid, e.g., Down's syndrome or Alzheimer's disease. Preferably, the cells used are neurons which produce intracellular β-amyloid (possibly in aggregated form), e.g., neurons from Down's syndrome patients (Yankner, B., Feb. 1997, Keystone Meeting "Molecular Mechanisms of Alzheimer's Disease), or guinea pig neurons treated with hydrogen peroxide (Younkin, S., Feb. 1997, Keystone Meeting "Molecular Mechanisms of Alzheimer's Disease). Preferably, the fluorescent ligand is a compound of formula **X** or a pharmaceutically acceptable salt thereof. More preferably, a dimeric fluorescent ligand is used, e.g., a compound of formula **IX** or a pharmaceutically acceptable salt thereof. Examples of fluorescent compounds include Congo Red fluorescein ligand **131**, Congo Red rhodamine ligand **133** and Congo Red coumarin ligand **135**. This method identifies agents which can penetrate the cell and reach the site of β-amyloid aggregation, as well as inhibiting the aggregation process itself.

The invention also includes a method for identifying a labeled ligand which selectively binds to one type of β-amyloid fibril. A labeled compound is provided. First β-amyloid fibrils are provided, and second β-amyloid are provided. The labeled compound is contacted with the first β-amyloid fibrils under conditions which allow the labeled ligand to interact

with the first β-amyloid fibrils. It is determined if the labeled compound binds to the first β-amyloid fibrils. If the labeled compound does not bind to the first β-amyloid fibrils, then the labeled compound is contacted with the second β-amyloid fibrils under conditions which allow the labeled compound to interact with the second β-amyloid fibrils. It is determined if the labeled compound binds to the second β-amyloid fibrils, binding being correlated with a labeled ligand which selectively binds to the second β-amyloid fibrils as compared to the first β-amyloid fibrils.

The binding of the labeled compound to the β-amyloid fibrils can be determined by standard methods known in the art, e.g., by retention of the labeled compound on a filter which retains the β-amyloid fibrils. Preferably, the labeled compound is a compound of formula I or pharmaceutically acceptable salts thereof, or formula II, III, IV or V. The β-amyloid fibrils can be any β-amyloid fibrils, including, e.g., protofibrils, type-1 fibrils, type-2 fibrils, neuritic plaque or diffuse amyloid. Preferred fibrils are composed of Aβ1-40 or Aβ1-42 proteins. In a preferred embodiment, the second β-amyloid fibrils are protofibrils. Labeled ligands which bind specifically to protofibrils can allow the non-invasive detection of protofibrils, the appearance of which can precede the appearance of neuritic plaque and Alzheimer's disease symptoms by years. Such a protofibril diagnostic is useful for identifying individuals for early therapeutic intervention.

The invention also includes the labeled ligand obtainable from this method.

The invention also includes a method for identifying a labeled ligand which binds to one or more amyloid proteins, e.g., β-amyloid, Islet amyloid polypeptide, Ig light chain, transthyretin, lysozyme, or β_2-microglobulin, using, e.g., a labeled compound of formula I, II, III, IV, V, or pharmaceutically acceptable salts thereof. The invention also includes the labeled ligand obtainable from this method.

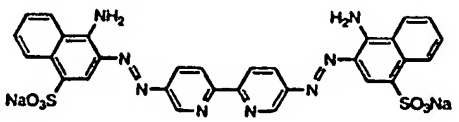
The following non-limiting examples further illustrate the present invention.

EXAMPLES

Example 1: Synthesis of Bipyridyl-Congo Red (2)

This example illustrates the synthesis of bipyridyl-Congo Red (2), represented by the following formula:

2



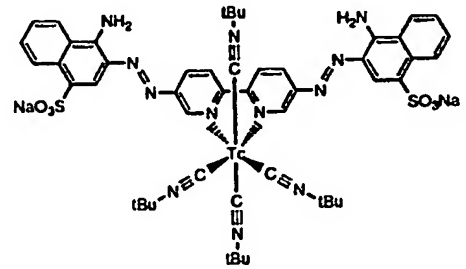
5,5'-diamino-2,2'-bipyridine was prepared from ethyl nicotinate according to the procedures described in Park, T.K. Ph.D. Thesis, Massachusetts Institute of Technology, Cambridge, MA, pp. 94-98, pp. 170-173 (1992); Knorpp et al., J. Nucl. Med. 1:23-30 (1960); Calogero et al., Dyes Pigm. 8:431-447 (1987). To a solution of 5,5'-diamino-2,2'-bipyridine dihydrochloride (75 mg, 0.30 mmol) in 10% HCl (420 μL) and H_2O (7.5 mL) at -5°C was added $NaNO_2$ (45 mg, 0.66 mmol) in H_2O (120 μL). After stirring at -5°C for 5 min, the resulting yellow solution was added to 4-amino-1-naphthalenesulfonic acid sodium salt (183 mg, 0.75 mmol) and sodium acetate trihydrate (324 mg, 2.4 mmol) in H_2O (3 mL) at -5°C. A distinct color change was immediately observed. After stirring at -5°C for 1 h, Na_2CO_3 (900 mg, 9mmol) was added. Purification by reversed-phase HPLC (H_2O/MeOH) afforded 114 mg (58%) of a purple solid.

Analytical HPLC; Delta-Pak C18 reversed-phase column (3.9 x 300 mm, 15-μm particle size, 300-Å pore size, Waters, Milford, MA); 15% MeOH/85% H_2O, 3 mL/min, R_v = 24 mL. ^1H NMR (CD_3OD) δ 8.82 (s, 2H), 8.46 (d, J = 7.3 Hz, 2H), 8.40 (s, 2H), 8.16 (d, J = 8.0, 2H), 8.03 (d, J = 8.0, 2H), 7.83 (d, J = 7.3, 2H), 7.21 (m, 4H); MALDI MS using TPKS (Juhasz and Biemann, Proc. Natl. Acad. Sci. U.S.A. 91:4333-4337 (1994)) for $C_{30}H_{22}N_8O_6S_2$ [M], calcd 654.7, found 654.6; UV (10 mM NaH_2PO_4, pH 7.4) λ_{max} 514 (ε = 2.78 x $10^4 cm^{-1} \cdot M^{-1}$, 344 ($\varepsilon$ = 2.21 x 10^4).

Example 2: Synthesis of a Technetium Complex with Bipyridal-Congo Red [Tc(CN*t*Bu)$_4$(bpcr)]$^+$(3)

This example illustrates the synthesis of a technetium complex (3) with bipyridal-Congo Red, represented by the following formula:

3



To a 4 mL vial was added 43 mM bipyridyl-Congo Red 2 (250 μL), obtained from Example 1, EtOH (500 μL), and 50 mM $NH_4[TcO_4]$ (400 μL, New England Nuclear, Boston, MA.). To this solution *tert*-butyl isocyanide (5 μL, 44 μmol) and 40 mM $Na_2S_2O_4$ (1.5 mL in aqueous NaOH at pH 12) were added, and the mixture was stirred and heated in

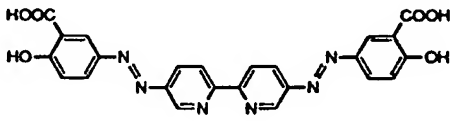
a boiling water bath for 1 h. The vial was then removed from the bath and allowed to cool to room temperature. Purification by flash chromatography using C18 corasil (37-50 μm, Waters, Milford, MA). (H_2O/MeOH) followed by reversed-phase HPLC gave 3.3 mg (28%) of a blue solid (specific activity = 1.26 MCI/mmol).

Analytical HPLC; 0-5 min 10% MeOH/90% H_2O, 5-20 min 10-100% MeOH, 3 mL/min, R_v = 48 mL. ^1H NMR (CD_3OD) δ 9.53 (s, 2H), 8.68 (d, J = 8.0, 2H), 8.58 (s, 2H), 8.13 (bs, 4H), 8.09 (d, J= 8.0, 2H), 7.47 (t, J = 8.2, 2H), 7.35 (t, J = 8.2, 2H), 1.70 (s, 18H) 1.38 (s, 18H); MALDI MS (Juhasz and Biemann, Proc. Natl. Acad. Sci. U.S.A. 91:4333-4337 (1994)) for $C_{50}H_{58}N_{12}O_6S_2Tc$ $[M]^+$, calcd 1085.3, found 1085.8; UV (10 mM NaH_2PO_4, pH 7.4) λ_{max} 546 (ε = 2.25 x 10^4), 332 (ε = 2.50 x 10^4).

Thus, the ^{99}Tc:bipyridyl ligand stoichiometries of the resultant complex were determined to be 1:1 by mass spectrometry, which showed parent ions for the complex, and by ^1H NMR, which showed two distinct resonances, corresponding to two equatorial and two axial t-butyl isocyanide ligands. (O'Connell et al., Inorg. Chem. 29:3539-3547 (1990)). The anticipated chelation of Tc by the bipyridal group (O'Connell et al., Inorg. Chem. 29:3539-3547 (1990); Adams et al., Inorg. Chem. 22:2798-2800 (1983)) was confirmed by ^1H NMR, which showed a retention of ligand symmetry and a downfield shift of the protons adjacent to the bipyridyl nitrogens in the complex 3(δ = 9.53) relative to the uncomplexed ligand 2(δ = 8.82).

Example 3: Synthesis of Bipyridal-Chrysamine G (5)

This example illustrates the synthesis of bipyridal-Chrysamine G (5), represented by the following formula:



5,5'-diamino-2,2'-bipyridine was prepared from ethyl nicotinate according to the procedures described in Park, T.K. Ph.D. Thesis, Massachusetts Institute of Technology, Cambridge, MA, pp. 94-98, pp. 170-173 (1992); Knorpp et al., J. Nucl. Med. 1:23-30 (1960); Calogero et al., Dyes Pigm. 8:431-447 (1987). To a solution of 5,5'-diamino-2,2'-bipyridine (50 mg, 0.27 mmol) in 10% HCl (1.34 mL) was added 2 M $NaNO_2$ (0.27 mL) slowly over 10 min at 0°C. The resulting solution was stirred for 1 h at 0°C during which

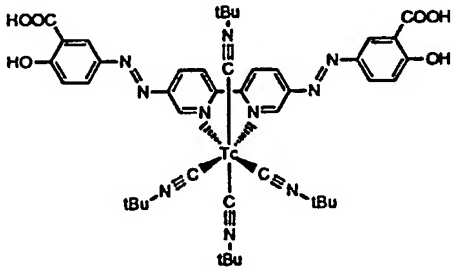
the color changed to pale yellow. To this solution at 0°C was added salicylic acid (111 mg, 0.81 mmol) in 0.5 M Na_2CO_3 (2.7 mL) which was cooled to 0°C prior to addition. The pH of the reaction mixture was adjusted to 9 by periodic addition of 0.5 M Na_2CO_3. After stirring overnight at 4°C, the reaction mixture was warmed to room temperature over 2 h, and acidified to pH 2 with 10% HCl. The precipitate was then filtered, washed with HCl (pH 2) twice, dried, washed with MeOH (2 x 10 mL), and finally washed with DMF (2 x 15 mL). The DMF solution was concentrated, filtered through a plug of silica gel with DMF, and dried *in vacuo* to provide 95 mg (73%) of a brown solid.

^{1}H NMR (DMSO-d_6) δ 9.16 (d, J = 2.2, 2H), 8.62 (d, J = 8.5, 2H), 8.34 (d, J = 2.7, 2H), 8.26 (dd, J = 8.5, 2.2, 2H), 7.87 (dd, J = 8.8, 2.7, 2H), 6.76 (d, J = 8.8, 2H); FABMS(+) for $C_{24}H_{15}N_6O_6[M - H]^+$, calcd 483.1, found 483.4; UV (DMF) λ_{max} 468 (ε = 2.67 x 10^4).

Example 4: Synthesis of a Technetium Complex with Bipyridal-Chrysamine G $[Tc(CNtBU)_4(bpcg)]^+$ (6)

This example illustrates the synthesis of a technetium complex (6) with bipyridal-Chrysamine G, represented by the following formula:

6



To a 20 mL hydrolysis tube charged with bipyridyl-Chrysamine G **5** (14.4 mg, 0.03 mmol), obtained from Example 3, H_2O (1.5 mL), and EtOH (1.5 mL) were added sequentially $NH_4[TcO_4]$ (0.6 mL of 30 mg/6 mL aqueous solution), tert-butyl isonitrile (15 μL), and $Na_2S_2O_4$ (30 mg dissolved in 3 mL H_2O) at room temperature. The reaction mixture was heated to 100°C for 1.2 h, cooled to room temperature over 1 h, and MeOH (10 mL) was added. The resulting suspension was filtered, concentrated to 1 mL, and ether (30 mL) was added. The precipitate was filtered, washed with excess ether to remove $[Tc(CNtBu)_6]^+$, and washed with MeOH (30 mL). The collected methanolic solution was concentrated, filtered through a plug of silica gel, and dried *in vacuo* to give 8 mg (29%) of

a blue-purple solid (specific activity = 0.51 mCi/mmol).

^{1}H NMR (CD_3OD) δ 9.52 (d, J = 2.1, 2H), 8.59-8.55 (m, 4H), 8.37 (dd, J = 8.4, 2.1, 2H), 7.99 (dd, J = 8.7, 2.4, 2H), 6.94 (d, J = 8.7, 2H), 1.64 (s, 18H), 1.28 (s, 18H); MALDI MS (Juhasz and Biemann, Proc. Natl. Acad. Sci. U.S.A. 91:4333-4337 (1994)) for $C_{44}H_{52}N_{10}O_6Tc$ $[M]^+$, calcd 915.4, found 915.9; UV (MeOH) λ_{max} 418 (ε = 2.39 x 10^4), 272 (ε = 1.21 x 10^4).

Thus, the ^{99}Tc:bipyridyl ligand stoichiometries of the resultant complex were determined to be 1:1 by mass spectrometry, which showed parent ions for the complex, and by ^{1}H NMR, which showed two distinct resonances, corresponding to two equatorial and two axial t-butyl isocyanide ligands. O'Connell et al., Inorg. Chem. 29:3539:3547 (1990). The anticipated chelation of Tc by the bipyridal group (O'Connell et al., Inorg. Chem. 29:3539-3547 (1990); Abrams et al., Inorg. Chem. 22:2798-2800 (1983)) was confirmed by ^{1}H NMR, which showed a retention of ligand symmetry and a downfield shift of the protons adjacent to the bipyridyl nitrogens in the complex (δ = 9.52) relative to the uncomplexed ligand **5**(δ = 9.16).

Example 5: Synthesis of Zinc, Cadmium, Nickel, Copper and Cobalt Complexes with Bipyridyl-Congo Red

This example illustrates the synthesis of zinc, cadmium, nickel and copper complexes with bipyridyl-Congo Red.

The stoichiometry of the metal complexes was determined from titrations performed under saturating conditions (the concentration of bipyridyl-Congo Red is much greated than K_d) where the stoichiometry of the complex was reflected by the number of equivalents of metal ions needed to reach saturation. Metal titrations were performed in a 1 cm path-length quartz cuvette with bipyridyl-Congo Red solutions (~20 μM) in buffer (10 mM NaH_2PO_4, pH 7.4) at 25°C. Spectra over the wavelength range 190-820 nm were collected after each addition of metal ions ($ZnCl_2$, $NiCl_2 \cdot 6H_2O$, $CoCl_2 \cdot 6H_2O$, $CuCl_2 \cdot 2H_2O$, and $CdCl_2 \cdot 2.5H_2O$) in 1 μM increments followed by equilibration with stirring for at least 30 min. Upon addition of metal ions to bipyridyl-Congo Red (**2**) the absorptions at 344 and 514 nm decreased in intensity with the concomitant appearance of two new absorptions at *ca.* 390 and 610 nm. Bipyridyl-Congo Red:metal stoichiometry determined at λ_{max}; Zn(II) (2:1 ratio, 610 nm), Ni(II) (2:1, 592), Cu(II) (3:1, 568), Cd(II) (2:1, 606), Co(II) (2:1, 608).

Example 6: Binding of Bipyridal-Congo Red Technetium Complexes to β-Amyloid

This example illustrates affinity of the radioactive complexes **3** and **6** for β1-40 amyloid fibrils. Bound and free ligand were separated by centrifugation. In both cases, saturable binding was observed. Saturation experiments for binding of **3** and **6** to β1-40 fibrils (5 μM total protein), as well as Scatchard analyses of the binding data, were performed.

Dissociation constants, IC_{50} and inhibition constants were determined. Aggregated β1-40 peptides (Bachem, Torrance, CA; peptide concentrations were determined by amino acid analysis) were prepared by stirring supersaturated solutions of peptide (100 μM) in the standard buffer (10 mM NaH_2PO_4, pH 7.4) at room temperature for at least 72 h and remained stirring while aliquots were taken. Equilibrium dissociation constants (K_d) were measured in standard buffer containing aggregated peptides (5.0 μM total β1-40) and the various concentrations of the labeled ligands. For determination of the IC_{50} value (concentration of inhibitor required to competitively decrease the fraction of the labeled ligand bound to fibrils by 50%), an aggregated peptide solution was added to a constant concentration of the labeled ligand and a varying concentration of inhibitor. The solution was briefly vortexed and allowed to equilibrate at room temperature for 2 h. The solution was centrifuged for 1 min at 14,000 rpm (16,000g) and the amount of the labeled ligand bound ([B]) to fibrils was obtained by measuring the radioactivity of the pellets or calculated using the equation [B] = [T] - [F] where [F] is the concentration of the labeled ligand in the supernatant (i.e., amount unbound or free) and [T] is the total amount of the labeled ligand added. A Scatchard plot was used to obtain $[B]_{max}$ (apparent maximum amount of the labeled ligand bound to fibrils) and K_d. (Freifelder, D., Physical Biochemistry: Applications to Biochemistry and Molecular Biology, 2nd ed.; W.H. Freeman & Co., N.Y. Chapter 10, p. 654 (1982)). The $[B]_{max}$ was based on total protein concentration. Given that not all of the protein is in the fibrous form (solubility of β1-40 is ca. 1 μM, NAC is ca. 11 μM), these values probably represent lower limits; the actual values may be higher. The IC_{50} value was used to calculate the inhibition constant K_i. (Cheng et al., Biochem. Pharmacol. 22:3099-3108 (1973)).

The K_d's of **3** and **6** were 630 nM and 160 nM, respectively (see Table 1).

Table 1: Dissociation Constants (K_d, μM) and Inhibition Constants (K_i μM) for β1-40 fibrils and NAC fibrils at 25°C and pH 7.4[a]

amyloid	Congo Red analogs	K_d	K_i[b]	K_i[c]
β1-40	$[Tc(CNtBu)_4(bpcr)]^+$(3)	0.63(0.06)		
	$[Tc(CNtBu)_4(bpcg)]^+$(6)	0.16(0.05)		
	Congo Red (1)		0.46(0.03)	0.56(0.14)
	Bipyridyl-CR (2)		0.51(0.11)	0.48(0.12)
	Bipyridyl-CG (5)		0.76(0.15)	0.42(0.10)
	$Zn(2)_2$		1.52(0.23)	
NAC	$[Tc(CNtBu)_4(bpcr)]^+$(3)	0.77(0.14)		
	$[Tc(CNtBu)_4(bpcg)]^+$(6)	0.43(0.15)		

[a] Data shown are an average of at least three separate experiments and are expressed as mean (±S.D.)
[b] This value was obtained using $[Tc(CNtBu)_4(bpcr)]^+$ as the labeled ligand.
[c] This value was obtained using $[Tc(CNtBu)_4(bpcg)]^+$ as the labeled ligand.

These values are comparable to the reported K_d's of Chrysamine G to β10-43 fibrils (Klunk et al., Neurobiol. Aging 15:691-698 (1994)) and of Congo Red to insulin amyloid fibrils (Klunk et al., J. Histochem. Cytochem. 37:1273-1281 (1989)).

The relative affinities (K_i's) of the free bipyridyl ligands were determined by displacement of complexes **3** or **6** (see Table 1). Comparable values were obtained for displacement of either complex, suggesting shared binding sites, which may be hydrophobic pockets spaced at regular intervals along the fibril surface. The ordered nature of these sites is suggested by the observed birefringent staining by Congo Red (Cooper, J.H., Lab. Invest. 31:232-238 (1974)), and the fact that the $Zn(2)_2$ complex, obtained from Example 5, in which the two bipyridyl Congo Red ligands are likely to be orthogonal, does not bind more tightly than the 1:1 Tc complexes (see Table 1). The stoichiometry of saturation, as estimated by Scatchard analysis of the binding curves, differed slightly, with 2.7 moles of **3** and 1.6 moles of **6** bound per mole of β1-40.

Example 7: Binding of Bipyridal-Congo Red Technetium Complexes to NAC

This example illustrates the binding of complexes **3** and **6** to amyloid fibrils comprising the minor brain amyloid peptide NAC. The NAC binding studies were done as described in Example 6 for β1-40, except the NAC concentration used was 15 μM. The binding showed similar features to the binding of β1-40 fibrils (see Table 1). Again, the

bipyridal Congo Red complex **3** bound less avidly than the bipyridal Chrysamine G complex **6**; dissociation constants of 770 nM and 430 nM, respectively, were measured as described in Example 6. Analogous to the case with β1-40 fibrils, the stoichiometry of saturation with **3** (1.4 moles **3** per mole NAC) was slightly higher than for **6** (0.6). The NAC amyloid fibrils and the β1-40 fibrils may share a general feature, suggested by their local sequence homology (Han et al., Chem. Biol. 2:163-169 (1995)), which is recognized by Congo Red, **3** and **6**.

Although complex **6** binds to both β1-40 and NAC amyloid fibrils two- to four-fold more avidly than does **3**, additional studies demonstrated that the relative affinity of two amyloid probes was not constant for a series of amyloid fibrils comprising different proteins. Thus, the ligands can show protein-specificity for different amyloids.

Example 8: Synthesis of Bisazo Linkers (L=L' = -N=N-)

A. Preparation of Tc-Complex (15) (Prep. Scheme I)

(i) 5,5'-Diethoxycarbonylamino-2',2'-bipyridine(9)

To diacid **7** (1.3 g, 5.32 mmol) in 20 mL of $SOCl_2$ was heated under reflux for 4 hr. The reaction mixture was concentrated in vacuo, suspended in 50 mL of acetone, and treated with 2.1 g of NaN_3 in 5 mL of H_2O in portions at 0°C. The mixture was stirred for 30 min, diluted with 100 mL of H_2O, filtered, thoroughly washed with H_2O, and dried in vacuo. The crude acyl azide **8** suspended in 50 mL of xylene and 50 mL of ethanol was heated under reflux overnight. The reaction mixture was cooled, and the white precipitate was filtered to give 1.5 g (85%) of dicarbamate **9** as white powder.

(ii) 5,5'-Diethoxycarbonylamino-6,6-difluoro-2'2'-bipyridine(10)

To bipyridine **9** (1.5 g, 4.5 mmol) were added 3 mL of H_2SO_4 and 3mL of fuming HNO_3 at 0°C. The mixture was heated at 90°C for 30 min. The reaction mixture was cooled and poured into crushed ice. The precipitated dinitrodicarbamate **10** was filtered off, washed with H_2O to give 1.3 g (68%) of product.

(iii) 5,5'-Diethoxycarbonylamino-6,6-difluoro-2'2'-bipyridine(11)

A solution of dinitrobipyridine **10** (0.5 g, 1.2 mmol) and 0.1 g of PtO_2 in ethanol (20 mL) was stirred under H_2 atmosphere for 30 min at room temperature. The reaction mixture was filtered off, concentrated in vacuo and suspended in 1 mL of 48% fluoboric acid. To

this solution was added a solution of sodium nitrite (0.18 g, 2.5 mmol) in water dropwise to maintain the reaction temperature around -5°C. After 1 hour, the reaction mixture was filtered off to give 0.17 g (40% yield) of difluoro-bipyridine **11**.

(iv) 5,5'-Diamino-6,6'-difluoro-2,2'-bipyridine(12)

A solution of dicarbamate **11** in 1:1 mixture of 2.5 M NaOH and DMSO was stirred overnight at 45°C, according to the procedure described in Andrews et al., J. Chem. Soc. PT1, 12:2995-3006 (1982). To the reaction mixture was added water, and the precipitated solid was filtered and washed with water to give the diamine **12**.

(v) Difluoro-azo dye (13)

Difluorodiamine **12** is tetrazotized by addition of $NaNO_2$. The resulting tetrazo salt is coupled with 2 eq. of salicylic acid to give the difluoro dye **13**.

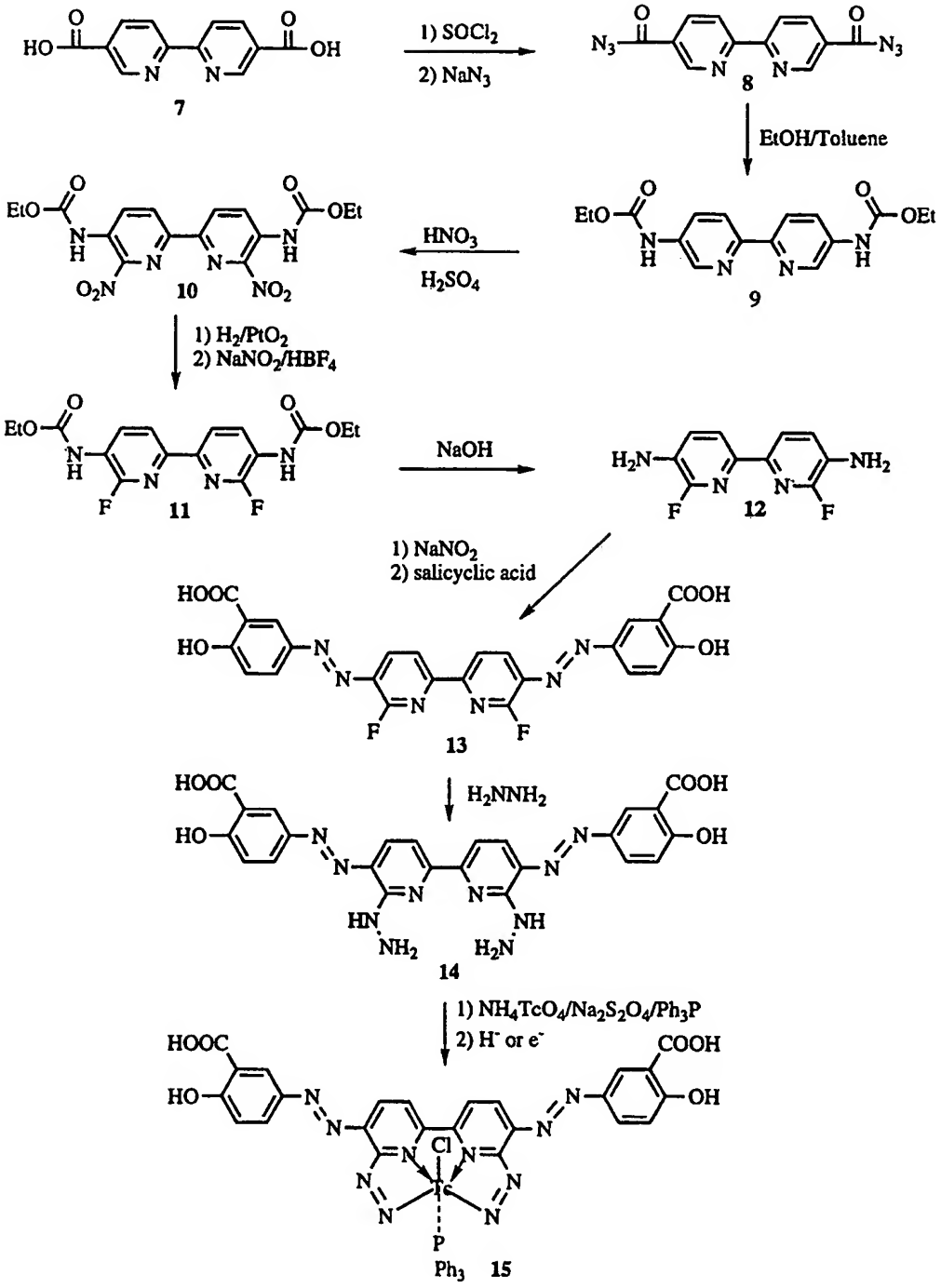
(vi) Dihydrazine-azo dye (14)

Following the procedure described in Ple et al., J. Heterocyclic Chem., 26:475-476 (1989), difluoride **13** in ethanol is treated with 2 eq. of hyrazine hydrate in ethanol at 0°C. After 1 hour, the dihydrazine dye **14** is isolated by filtration and purified by column chromatography.

(vii) Tc-Complex (15)

A mixture of the dye **14**, 2 eq. HCl, NH_4TcO_4, and $Na_2S_2O_4$ in EtOH is heated under reflex for 6 to 12 hours, and cooled to room temperature. The precipitated solid is removed, dissolved in EtOH, treated with 1 eq. triphenyl phosphine, and the resulting Tc^+ is further reduced to Tc° either electrochemically or with metal hydrides. The same complex **15** can be made from a photochemical reaction of the dye ligand **14** with hexakis-tbutylisonitrile-Tc complex.

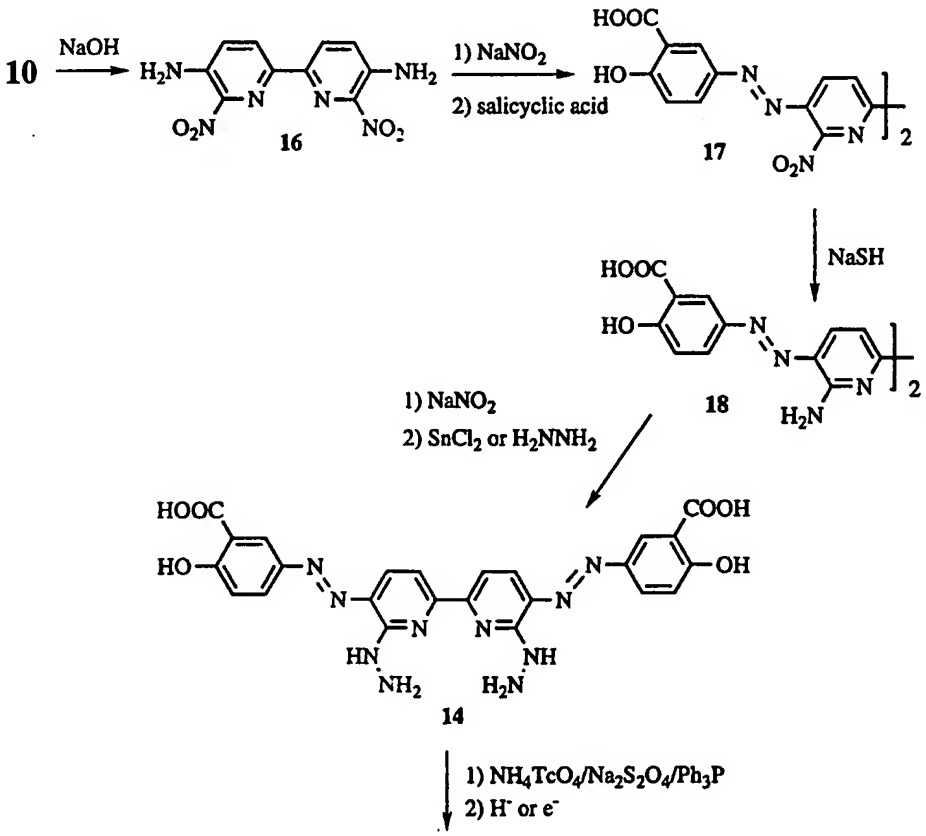
PREP. SCHEME I



B. Alternative Preparation of Tc-Complex (15) (Prep. Scheme II)

Dinitro-diamine **16**, prepared from **10** via hydrolysis, is tetrazotized and coupled with salicylic acid. The resulting dinitro azo dye **17** is reduced to diamine with H_2/PtO_2 in ethanol or preferentially with NaSH according to the procedure described in Ueno, J. Amer. Chem. Soc. 74:4508-4511 (1952). The diamine **18** is then tetrazotized and reduced with $SnCl_2$ or Zn to dihydrazine **14** (Hass et al., J. Org. Chem. 15:8-14 (1950)). When the reduction of diazonium group to hydrazine is problematic, direct substitution of hydrazine provides the same product **14**, which is transformed to the complex **15** under the same protocol as described above.

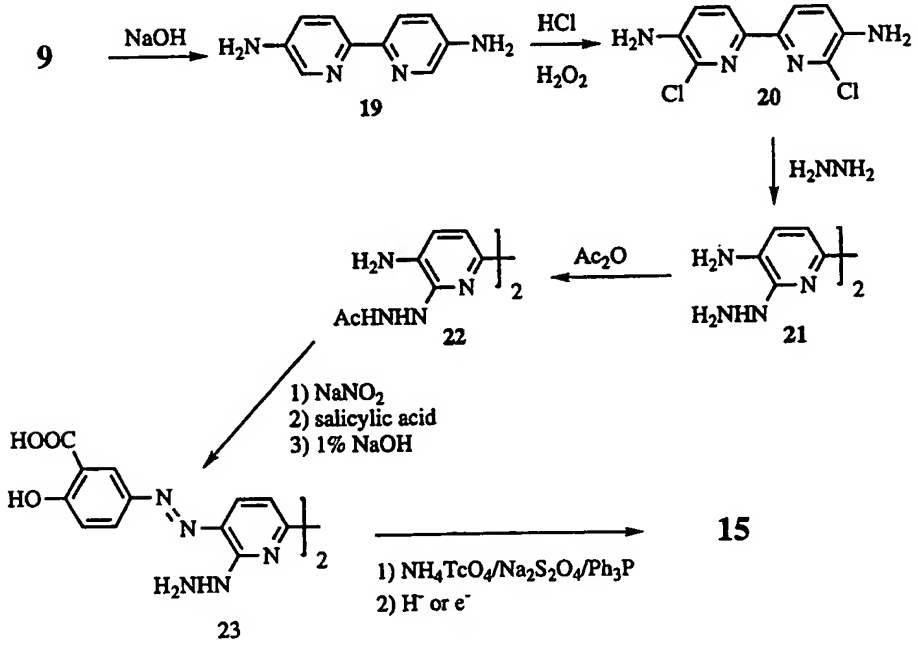
PREP SCHEME II



C. Alternative Preparation of Tc-Complex 15 (Prep. Scheme III)

The diamide 9 was hydrolyzed to the diamine 19 according to Andrews et al., J. Chem. Soc. PT1, 12:2995-3006 (1982). To 0.5 g of diamine 19 was added 2.5 mL of 35% HCl, and the solution was heated at 80°C. To this solution was added 0.4 mL of 38% H_2O_2, and the resulting solution was stirred for 5 hours at 80°C. The reaction mixture was cooled to room temperature, and basified with 2.5 M NaOH. The brown solids precipitated was filtered off and dried in vacuo. A mixture of crude 20 and hydrazine in DMSO is heated overnight to give the diaminodihydrazine 21. The dihydrazine 21 is treated with 2 eq. Ac_2O to the protected hydrazine 22, which is then tetrazotized, coupled wtih salicylic acid, and deprotected to afford the dye 23. The dye 23 is transformed to the complex 15 using the same protocol.

PREP SCHEME III

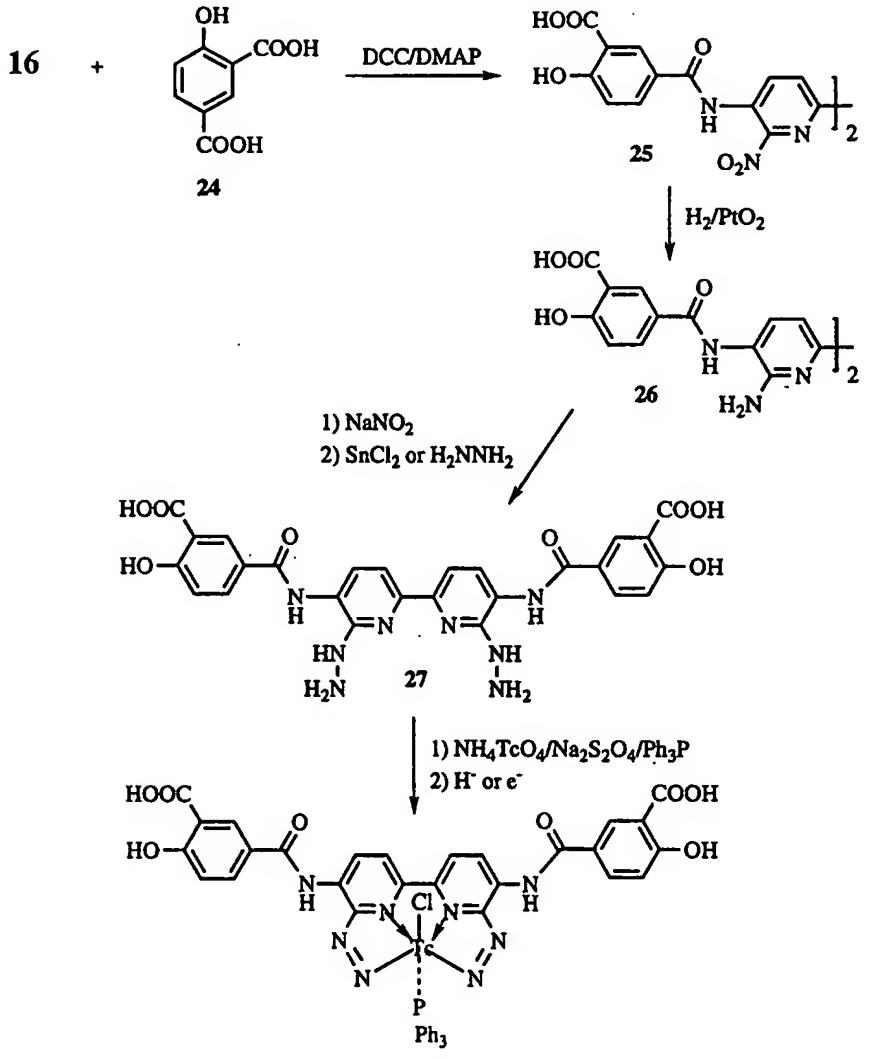


Example 9: Synthesis of Bisamide Linkers (L=L' = -CONH-)

Preparation of Complex 28 (Prep. Scheme IV)

Diamino-dinitro-bipyridine **16** is coupled with 4-hydroxyisophthalic acid (**24**) using DCC/DMAP or other coupling conditions (Klausner et al., Synthesis, 549-559 (1974)), to provide diamide **25**. The dinitrodiamide **25** is reduced with H_2/PtO_2 to diamine **26** as described above. The diamine **26** is tetrazotized and treated with hydrazine or reduced with stannous chloride to the diamide ligand **27**. The bis-amide dye **27** is transformed to the Tc-complex **28** using the same protocol as for **15**.

PREP SCHEME IV

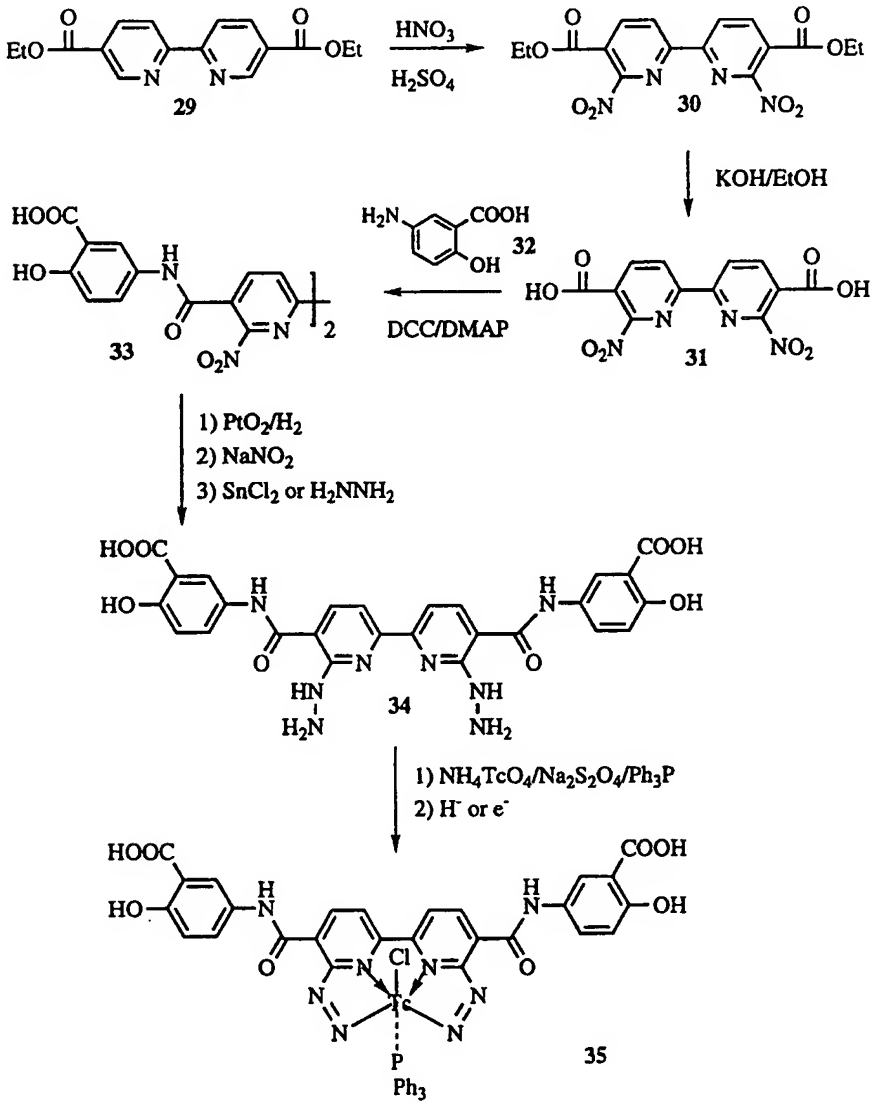


Example 10: Synthesis of Bisamide Linkers (L=L' = -NHCO-)

Preparation of Bisamide 35 (Prep. Scheme V)

Diethyl-bipyridine-dicarboxylic acid **29** is nitrated with H_2SO_4/fuming HNO_3 to provide the dinitro ester **30**. The ester **30** is saponified with KOH/EtOH to the diacid **31**, which is then coupled with 4-aminosalicylic acid (**32**) under DCC/DMAP condition to give diamide **33**. The nitro groups in **33** are reduced to amines by catalytic hydrogenation, and the resulting diamine is tetrazotized, reduced with stannous chloride or treated with hydrazine to afford the bisamide ligand **34**. The bis-amide dye **34** is transformed to the Tc-complex **35** using the same protocol as for **15**.

PREP SCHEME V

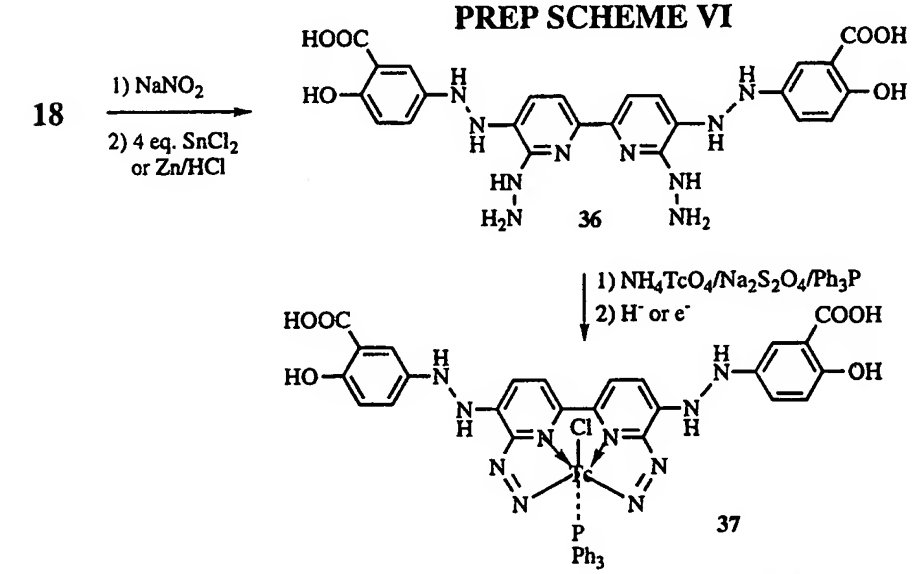


Example 11: Synthesis of Bishydrazine Linker (L=L' = -HN-NH-)

Preparation of Complex 37 (Prep. Scheme VI)

Bisazodiaminobipyridine **17** is tetrazotized with $NaNO_2$ and reduced with 4 eq. of $SnCl_2$ or Zn/HCl to the corresponding bishydrazinodihydrazine ligand **36**. The bis-hydrazide **36** is transformed to the Tc-complex **37** using the same protocol as for **15**.

PREP SCHEME VI

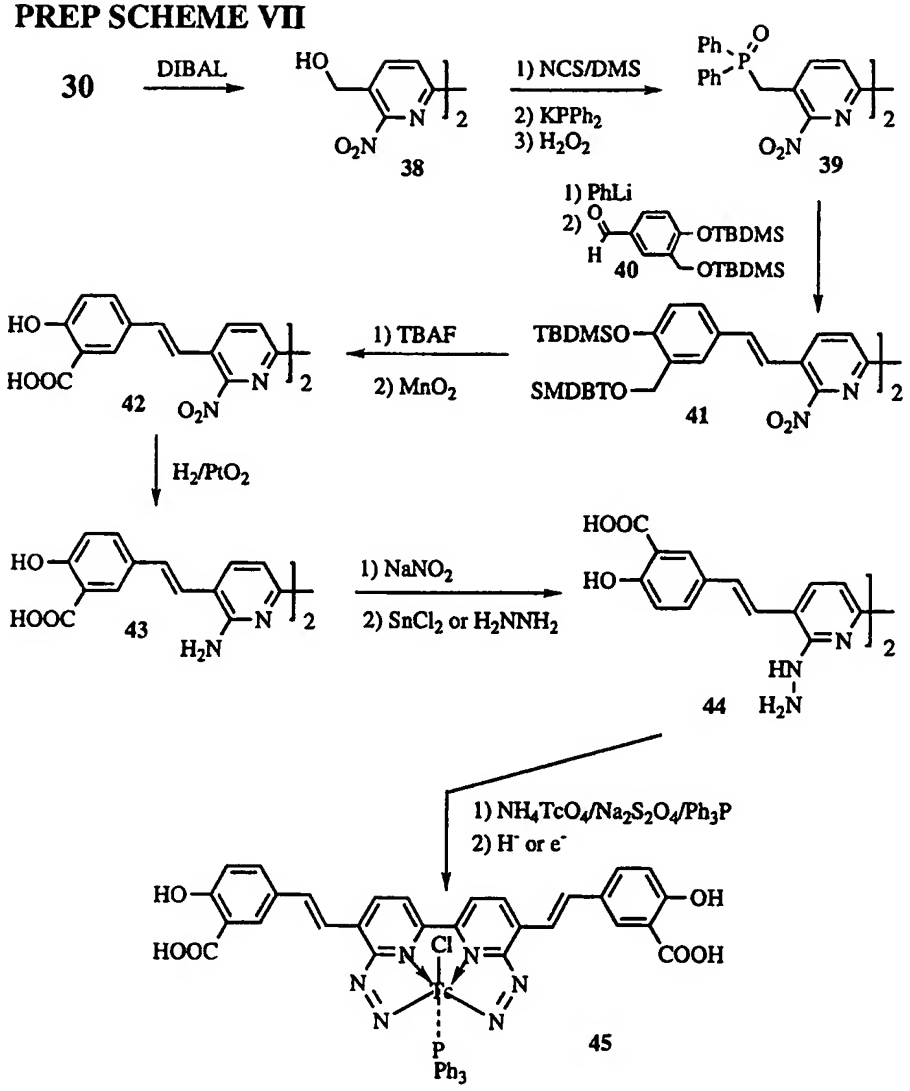


Example 12: Synthesis of Bisalkene Linker (L-L' = -C=C-,TRANS)

Preparation of Bisalkene 45 (Prep. Scheme VII)

The diester **30** is reduced to the diol **38** with DIBAL (diisobutylaluminum hydride). The diol groups in **38** are subsequently treated with first a mixture of NCS (N-chloro succinimide) and DMS (dimethyl sulfide), second with potassium diphenylphosphide, and finally with hydrogen peroxide to afford the phosphine oxide **39** (Posner et al., J. Org. Chem., 60:4617-4628 (1995)). The phosphine oxide **39** is deprotonated with phenyl lithium, and coupled with the aldehyde **40** (Lythgoe et al., J. Chem. Soc. PT1, 6:590-595 (1978)), prepared from 4-hydroxy-3-methyl benzaldehyde after hydroxylation with SeO_2 and followed by protection with TBDMS (t-butyldimethylsilyl) groups. The bis-alkene **41** is treated with TBAF (tetrabutylammonium fluoride) to unmask the alcohol groups. The benzylic alcohol in **41** is oxidized with MnO_2 to the corresponding carboxylic acids (Ahrens et al., J. Heterocyclic Chem., 4:625-626 (1967)). Platinum oxide catalyzed reduction of dinitro groups in **42** provides the diamine **43** which is then tetrazotized with sodium nitrite and reduced with stannous chloride or treated with hydrazine to give the bis-alkene ligand **44**. The bis-alkene **44** is transformed to the Tc-complex **45** using the same protocol as for **15**.

PREP SCHEME VII



Example 13: Synthesis of R=R'= $-CH_2OH$, $-CH_2NH_2$, $-CH_2SH$, $-o-C_6H_4-CH_2-COOH$

A. Preparation of Complexes **52**, **53**, and **54** (Prep. Scheme VIII)

(i) Tc-Complex **52**

To the diamine **20** in 5% NaOH is added 2 eq. of Boc_2O at room temperature to give the dicarbamate **46**. The dicarbamate **46** is treated with 4 eq. of nBuLi at -20°C, and quenched with DMF at -20°C. After 2 hours at room temperature, the bis-alcohol **47** in the reaction mixture is treated with 2 eq. of benzoyl chloride to give the bis-benzylester **48**. The diamine **48** is treated with $NaNO_2$, and coupled with salicylic acid to afford the hydroxy dye **49**. A mixture of the dye **49**, ammonium pertechnetate, dimercaptoethane, and sodium dithionate in EtOH is heated under reflux to give the Tc complex **52**.

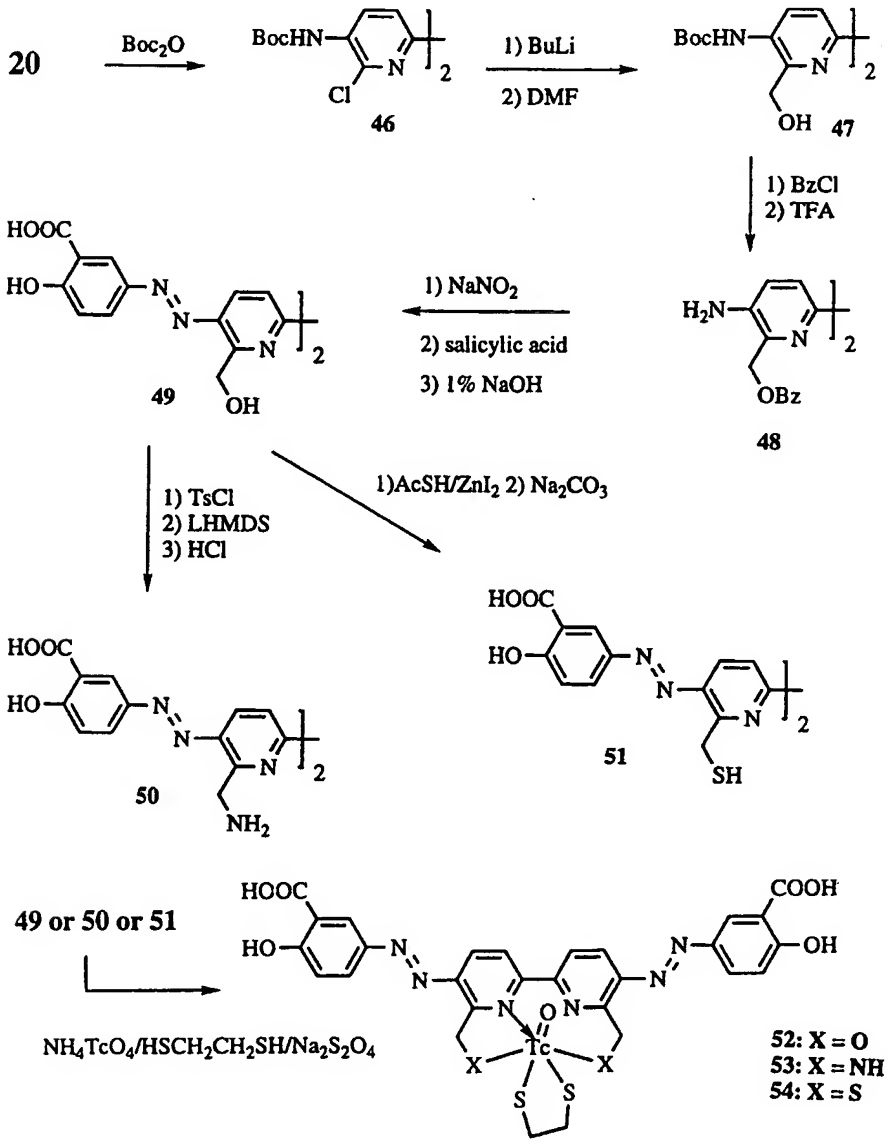
(ii) Tc-Complex 53

The bis-alcohol **49** is transformed to the bis-amine **50** via successive treatments with p-toluenesulfonyl chloride, lithium bis(trimethylsilyl)amide, and finally with hydrochloric acid (Mukaiyama et al., Tetrahedron Lett. 39:3411-3414 (1970). The dye **50** is similarly transformed to the complex **53** using the same protocol as for **52**.

(iii) Tc-Complex 54

The bis-alcohol **49** is treated with thiolacetic acid and ZnI_2 (Gauthier et al., Tetrahedron Lett. 27:15-18 (1986)), to give the bis-thiol **51** after unmasking step. The dye **51** is similarly transformed to the complex **54** using the same protocol as for **52**.

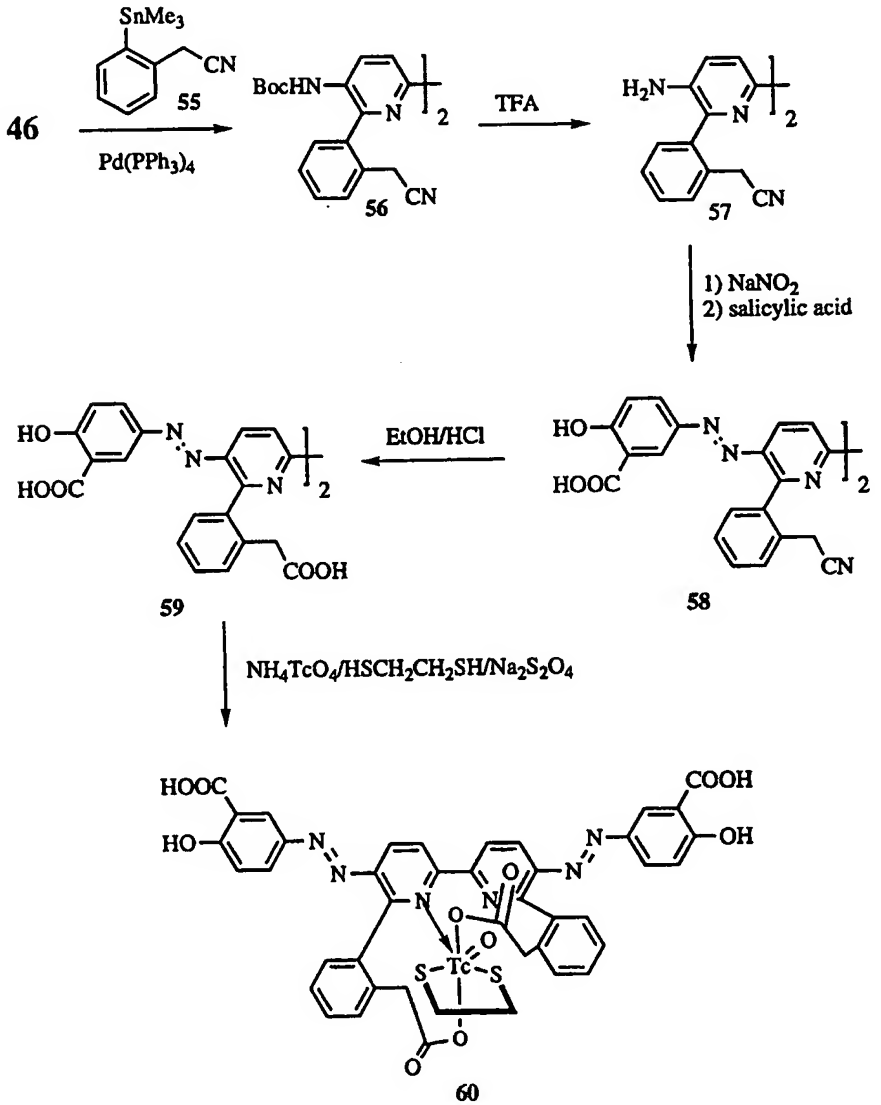
PREP SCHEME VIII



B. Preparation of Tc-Complex 60 (Prep. Scheme IX)

The dichloride **46** is reacted with the tin compound **55**, prepared according to the procedure described in Bates et al., Tetrahedron Lett., 37:267-270 (1996), in the presence of tetrakis-(triphenylphosphine)-palladium to give the adduct **56**. The bis-carbamate **56** is treated with trifluoroacetic acid (TFA) to afford the diamine **57**, which is tetrazo coupled with salicylic acid to give the dye **58**. The nitrile groups in the dye **58** are hydrolyzed to the dicarboxylic acid **59**, which is then transformed to the complex **60** using the same protocol as for **52**.

PREP SCHEME IX

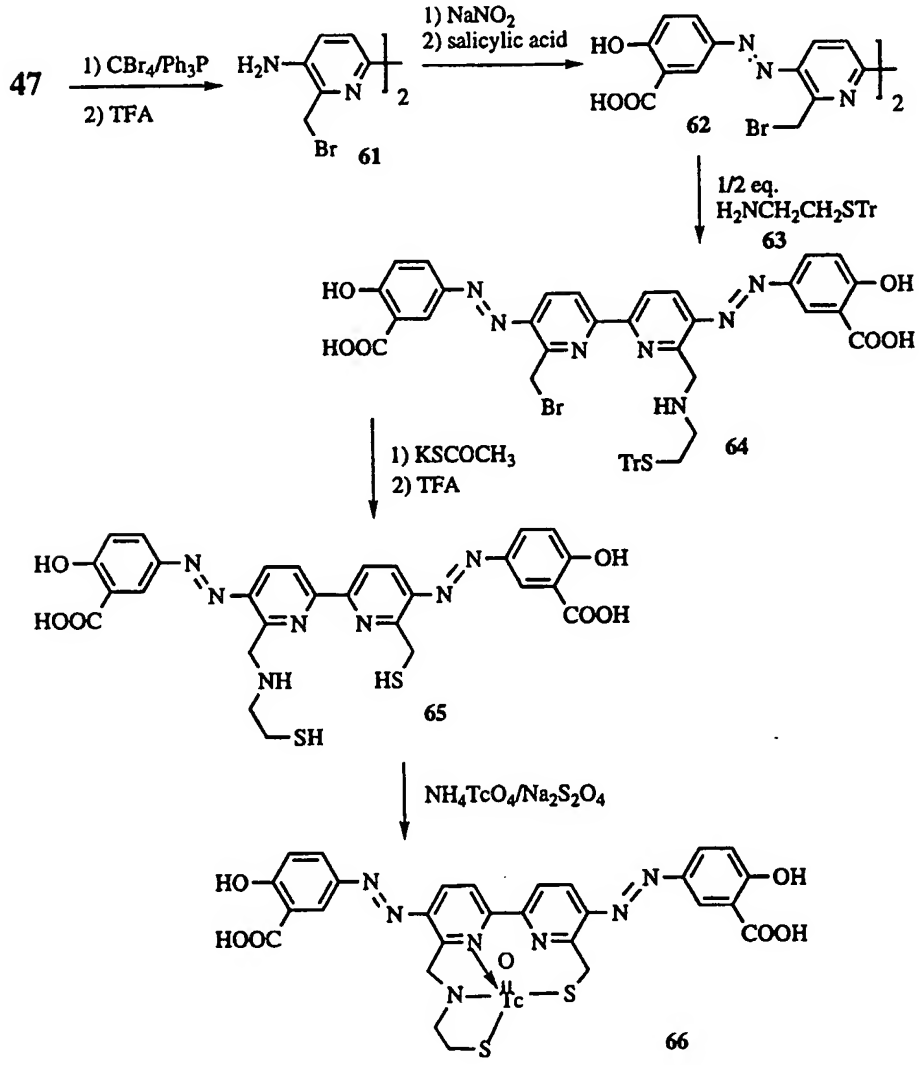


Example 14: Synthesis of R= $-CH_2NHCH_2CH_2SH$, R'= $-CH_2SH$

Preparation of the Tc-Complex 68 (Prep. Scheme X)

The bis-alcohol **47** is brominated on the benzylic positions with CBr_4/PPh_3 system to the bis-bromide **61**. Boc groups in **61** are removed with TFA to the corresponding bis amine, tetrazotized, coupled with salicylic acid to the dye **62**. The displacement of one bromide group in the dye **62** is proceeded by using 0.5 eq. of the amine **63**, prepared according to Hiskey et al., J. Org. Chem. 31:2178-2183 (1966). The other bromide group in **64** is then displaced with potassium thiolacetate, treated with TFA to the dithiol **65**. The dye **65** is suspended in EtOH and mixed with ammonium pertechnetate and sodium dithionate, and the resulting solution is heated under reflux to give the Tc-oxo complex **66** (Madras et al., Synapse 22:239-246 (1996)).

PREP SCHEME X



Example 15: Imaging with Organometallic Ligands Which Can Cross the Blood Brain Barrier Alone

This example illustrates administration and imaging using an organometallic ligand which can cross the blood brain barrier by itself. The procedure used is adapted from Madras et al., Synapse 22:239-246 (1996). The organometallic ligand, e.g., **54** or **66** (ca. 15-75 mCi per 65 kg body weight) is administered to a patient intravenously via catheter as a saline solution. Images are acquired after 2 hours using a digital ASPECT system (Digital Scintigraphics, Inc., Boston, MA) or a comparable instrument (see, e.g., Neuwalt, Implication of the Blood Brain Barrier and Its Manipulation, Vol. II, Plenum Press, N.Y. (1989) p. 210). The procedure for reconstruction is described in Holman et al., J. Nucl. Med., 31:1206-1210 (1994).

Example 16: Imaging with Organometallic Ligands Plus a Blood Brain Barrier Opener

This example illustrates administration and imaging using an organomettalic ligand plus alternative blood brain barrier openers.

Method 1: Osmotic Blood Brain Barrier Modification with Mannitol

The procedure used is adpated from Neuwalt, Implication of the Blood Brain Barrier and Its Manipulation, vol. II, Plenum Press, N.Y. (1989), pp. 207-213. The blood brain barrier is reversibly disrupted by administration of an arterial bolus of a hypertonic mannitol solution. This procedure has been used extensively to deliver drugs and imaging agents to the brain. A 10-step procedure is given in Neuwalt, vol. II, p. 213 (steps 5, 10-13 are specific to brain tumor patients).

After anesthetizing the patient, a hypertonic mannitol solution (180-300 ml over 30 sec.) is injected arterially, for example, via the vertebral artery or cartoid artery (see Neuwalt, p. 213). After about 5 minutes to about 3 hours, 15-30 mCi of the organometallic ligand, e.g., **3** or **6**, is administered intravenously. Imaging is done as described in Neuwalt, p. 210. The radioactivity is quantified and localized. Patients in the early stages of Alzheimer's disease localize a significantly increased amount of radioactive ligand relative to age-matched controls. Late-stage Alzheimer's disease patients localize an even greater amount of radioactivity.

Method 2: Opening of the Blood Brain Barrier with Organic Solvents

Dimethyl sulfoxide (DMSO) is used to reversibly and innocuously open the blood brain barrier. The procedure used is adapted from Neuwalt, Implication of the Blood Brain Barrier and Its Manipulation, vol. I, Plenum Press, N.Y. (1989) pp. 336-337, and is modified, replacing the antitumor compound with 15-75 mCi of the organometallic ligand, e.g., **3** or **6**.

Method 3: Opening of the Blood Brain Barrier with Drugs

Two classes of drugs have been shown to reversibly open the blood brain barrier. See Neuwalt, Implication of the Blood Brain Barrier and Its Manipulation, vol. I, Plenum Press, N.Y. (1989) pp. 332-334 and 337. Metrazol, a CNS stimulant, reversibly opens the barrier. This drug is coadministered with an anti-seizure drug, in order to suppress seizures which can result from the effective dose. In addition, certain anticancer drugs open the blood brain barrier for a relatively long period of time. These compounds, however, are toxic, and thus can cause additional problems. 15 - 30 mCi of the organometallic ligand, e.g., **3** or **6**, is injected into the patient subsequent to administration of the drug.

Method 4: Opening of the Blood Brain Barrier with RMP-7

Polypeptides called receptor mediated permeabilizers (RMP) have been shown to increase the permeability of the blood brain barrier to various agents. See U.S. Patent No. 5,268,164. RMP-7 is used according to the procedure described in U.S. Patent No. 5,268,164 (column 14), so as to open the blood brain barrier and allow the organometallic ligand, e.g., **3** or **6**, to pass through.

Example 17: Organometallic Ligands as Therapeutics

This example illustrates treatment of Alzheimer's disease with an organometallic ligand so as to inhibit aggregation of amyloid proteins in the brain. The organometallic ligand, e.g., Zn complex analogous to **3**, at a dose of 10 mg/kg/day, is administered intravenously to a patient having Alzheimer's disease. A blood brain barrier opener is coadministered or preadministered (see Example 16), if necessary, to allow crossing of the blood brain barrier by the organometallic ligand. This procedure is followed daily for 100 days. Amyloid formation is imaged by the method described above. As a result of such treatment, aggregated amyloid formation is reduced.

Example 18: Detection of Scrapie Prion in Hamster Brain Tissue

This example illustrates a method for detecting the presence of scrapie prion in hamster brain tissue using an organometallic ligand. Hamster brain tissue (0.2g) from normal and scrapie hamsters (obtainable from NIAID, Rocky Mountain Laboratory, Hamilton, MT) is homogenized in water (2mL). An aliquot of the homogenate (5 μL) is removed and diluted into 100 μL with a 4% methanol-water solution of the ^{99}Tc-Congo Red complex (15 μM), e.g., **3** or **6**. The mixture is incubated for 30 min. at 25°C, and then spun for 30 min. at 234,000 x g at 20°C. The supernatant (95 μL) is separated from the pellet (5 μL) by pipetting. The two fractions are separately analyzed by scintillation counting, and the ratio of pellet cpm/supernatant cpm is determined. PrP^{Sc} (hamster) in scrapie hamster brain is detected above background.

Example 19: Detection of Bovine Spongiform Encephalopathy Prion (PrP^{Sc}) in Bovine Tissue

This example illustrates a method for detecting the presence of BSE prion in bovine brain or lymph tissue using an organometallic ligand. Bovine brain (est. 3-300 μg PrP^{Sc}/g diseased brain), or lymph tissue (*ca.* 10 mg), is homogenized in water (100 μL). An aliquot of this homogenate (5 μL) is removed and diluted to 100 μL with a 4% methanol-water solution and the mixture is incubated for 30 min. at 25°C, then spun for 30 min. at 234,000 x g at 20°C. The supernatant (95 μL) is removed by pipetting and the pellet is resuspended in 95 μL of a 10% w/v solution of N-lauryl sarcosinate to solubilize membrane-associated proteins and the centrifugation procedure is repeated. The pellet from the second centrifugation is diluted into 95 μL of 4% methanol-water containing *ca.* 150 nM ^{99m}Tc-Congo Red (**3**). The sample is sedimented as above and the pellet and supernatant are analyzed by a γ-camera. The ratio of cpm pellet/cpm supernatant is quantified; a high ratio indicates the presence of PrP^{Sc}. The amount of PrP^{Sc} in the pellet is determined by comparison of the pellet cpm to a standard curve, determined using purified PrP^{Sc}.

Example 20: Preparation of NX_3 Complex **120** (Prep. Scheme XI)

(i) 5-Bromo-2-N-Boc -Amino-Benzyl Alcohol **111**

To a flask charged with 2.15 g (10 mmol) of 5-bromo, 2-amino-benzoic acid **109** was

added 60 mL of THF and 1.7 g of NaH (60% in mineral oil) at room temperature. The mixture was heated under reflux for 1 hr, cooled to room temperature. To the mixture was added 2.04 g (1.2 eq) of di t-butyl dicarbonate and the resulting mixture was refluxed for 30 min, cooled to room temperature, and 0.8 g of NaH was added into the mixture. The reaction mixture was refluxed for 16 hr under Ar atmosphere. The mixture was then quenched with water carefully, acidified to pH 3, extracted with methylene chloride, concentrated to give the crude acid **110** (used for the next reaction without further purification). To a solution of **110** in 50 mL of THF was added N-methylmorpholine (1.0 g) and chloroethylformate (1.1 g) at -10°C. After 10 min, $NaBH_4$ (1.1 g) was added all at once, followed by careful addition of 100 mL of anhydrous MeOH with ice cooling. After 1 h, the reaction mixture was concentrated and partitioned into water and ethyl acetate. The separated organic layer was dried over $MgSO_4$, concentrated, and chromatographed to give the alcohol **111** (1.4 g, 47 % from **109**) as a colorless solid. ^1H NMR (300 MHz, $CDCl_3$) δ 7.81 (d, 1H, J = 8.7 Hz), 7.65 (bs, 1H), 7.38 (dd, 1H, J_1 = 8.7, J_2 = 2.4), 7.27 (d, 1H, J = 2.4), 4.62 (d, 2H, J = 5.9), 1.50 (s, 9H).

(ii) 5-Bromo-2-N-Boc-Amino-Acetyl Benzoate **112**

To a solution of the alcohol **111** (1.24 g, 4.13 mmol) in 20 mL of anhydrous methylene chloride, 1 mL of acetic anhydride, 1 mL of acetyl chloride and 1 mL of pyridine was added 4-N,N-dimethylaminopyridine (10 mg) at room temperature. After 1 hr, the reaction mixture was partitioned into methylene chloride and water. The separated organic layer was dried over $MgSO_4$, concentrated and chromatographed to give **112** as a white solid (1.40 g, 4.08 mmol, 99% yield). ^1H NMR (300 MHz, $CDCl_3$) δ 7.78 - 7.76 (m, 1H), 7.45 - 7.42 (m, 2H), 5.03 (s, 2H), 2.10 (s, 3H), 1.52 (s, 9H).

(iii) 5-Bromo-2-N-Boc-Amino-Benzaldehyde **114**

To a flask charged with the alcohol **111** (6.6 mmol) in 10 mL of methylene chloride was added 0.12 mL of DMSO, oxalyl chloride (0.07 mL) and 0.5 mL of triethylamine at -78°C. After 3 hr, the reaction mixture was diluted with ether and washed with aqueous $NaHCO_3$, water, and brine. The organic phase was dried over $MgSO_4$, concentrated and the crude product was purified by silica gel chromatography (solvent system) to afford aldehyde **114** as a white solid (0.17 g, 5.69 mmol, 86% yield). ^1H NMR (300 MHz, $CDCl_3$) δ 10.29 (bs, 1H), 9.82 (s, 1H), 8.93 (d, J = 9.0 Hz), 7.71 (d, 1H, J = 2.3), 7.63 (dd, 1H, J_1 = 9.0, J_2 = 2.3), 1.52 (s,9H).

(iv) Stannylated Benzoate **113**

A mixture of 1.33 g (3.88 mmol) of the bromide **112**, 0.24 g (0.2 mmol) of tetrakis (triphenylphosphine) palladium, 2.58 g of bistributyltin in 40 mL of triethylamine was refluxed under Ar for 12 hr. After cooling, the reaction mixture was decanted and chromatographed (95/5 hexane/ethyl acetate) to givethe stannyl benzoate **113** as a colorless oil (1.03 g, 1.86 mmol, 48% yield). ^{1}H NMR (300 MHz, $CDCl_3$)

(v) Biphenyl Adduct **115**

A mixture of the 0.17 g (0.57 mmol) of the bromo benzaldehyde **114**, 0.13 g (0.1 mmol) of tetrakis triphenylphosphine palladium in 10 mL of anhydrous toluene was refluxed under argon for 2 hours. A solution of the stannyl benzoate **113** in 5 mL of anhydrous toluene was added dropwise through the syringe over 1 hr while keeping the reaction mixture under reflux. After 12 h, the reaction mixture was concentrated and the crude product was purified by silica gel chromatography to give 0.11 g of the product **115** (0.11 g, < 43 % yield) contaminated with trace of tributyltin compounds. ^{1}H NMR (300 MHz, $CDCl_3$) δ 10.09 (bs, 1H), 9.98 (s, 1H), 8.52 (d, 1H, J = 8.3), 7.96 (d, 1H, J = 8.3), 7.78 - 7.76 (m, 2H), 7.55-7.53 (m, 2H), 5.17 (s, 2H), 2.12 (s, 3H), 1.55 (s, 9H).

(vi) Biphenyl Methoxybenzyl Sulfide **116**

A solution of 80 mg of the biphenyl adduct **115** in 3mL of THF and 1mL of water was treated with excess LiOH at 0°C. After 6 hr, the reaction mixture was neutralized with aq. HCl, extracted with methylene chloride (30 mL x 2), dried over $MgSO_4$, and concentrated. The crude product was purified by silica gel chromatography to provide the biphenyl alcohol (0.06 g). To a solution of the alcohol in 5 mL of THF was added 50 mg of carbon tetrabromide and 40 mg of triphenylphosphine at room temperature. After 2 hr, the reaction mixture was treated with the THF solution of the sodium thiolate prepared from 46 mg of 4-methoxy benzyl mercaptan and 200 mg of sodium hydride (60% in mineral oil) at room temperature. After 2 hr, the reaction mixture was partitioned into ethyl acetate and water. The separated organic layer was dried over $MgSO_4$, concentrated and purified by silica gel chromatography to afford the thio-aldehyde **116** (55 mg, 70% overall yield from **115**).

(vii) Biphenyl Bromide **117**

A solution of 55 mg (0.095 mmol) of the aldehyde **116** in 5 mL of anhydrous MeOH was treated with 20 mg of $NaBH_4$ at room temperature. After 1 hr, the reaction mixture was quenched with 5 mL of water, extracted with ethyl acetate, dried over $MgSO_4$, concentrated, dissolved in 5 mL of THF and treated with carbon tetrabromide and triphenyl phosphine at

room temperature. After 1 hr, the reaction mixture was concentrated and the crude product was purified by silica gel chromatography to afford the bromide **117**.

(viii) Biphenyl Bis-Hydroxyethyl Amine **118**

To a solution of the bromide **117** in dioxane and water was added 2 eq. of bis-ethanolamine and solid Na_2CO_3 at room temperature. After 12 h, the reaction mixture was extracted with methylene chloride, dried over $MgSO_4$, and concentrated. The crude product was purified by silica gel chromatography to afford the bis-hydroxyethyl amine **118** (18 mg, 0.027 mmol, 42% overall yield from **116**).

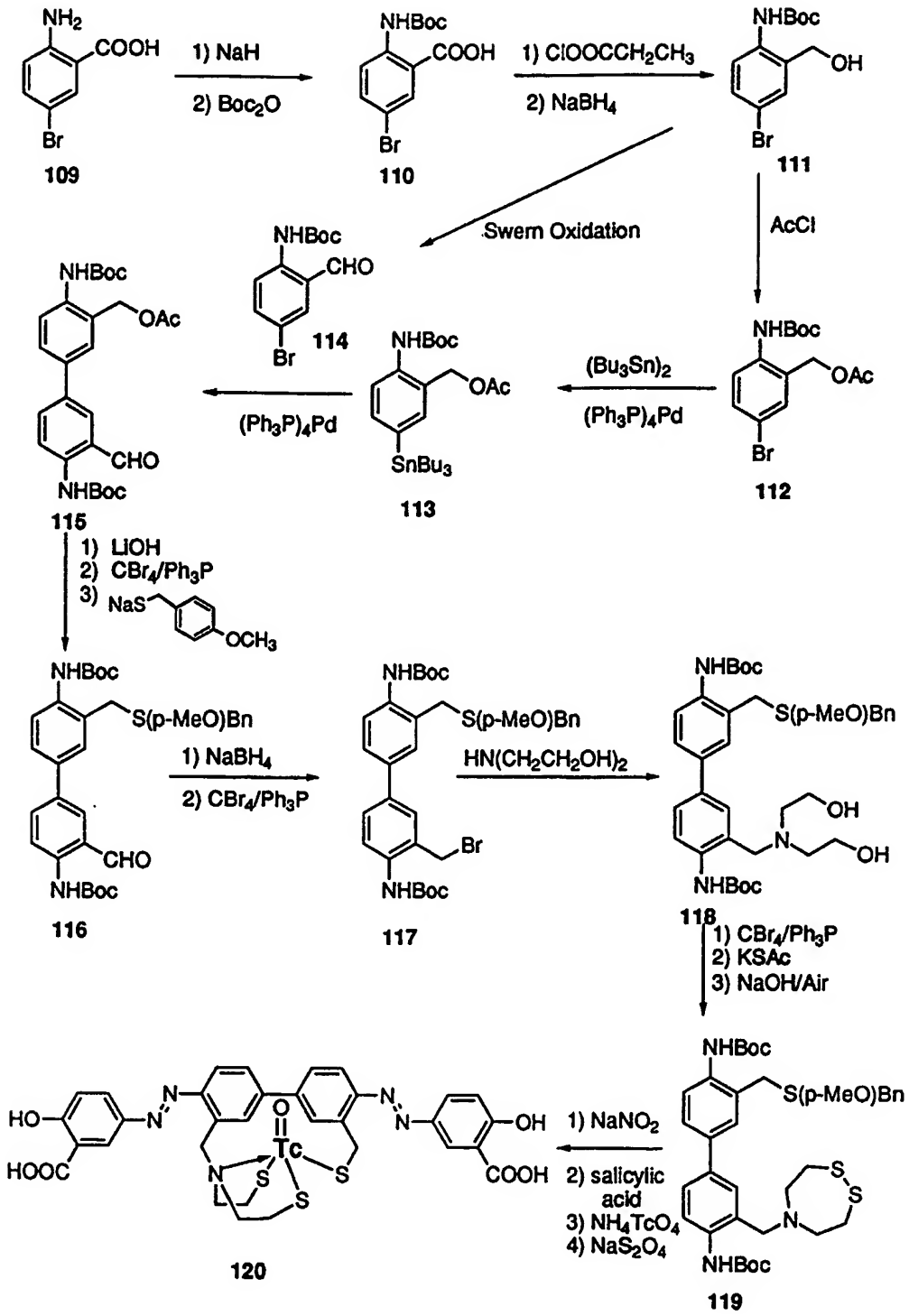
(ix) Biphenyl Disulfide **119**

A solution of 15 mg (0.023 mmol) the bis-hydroxyethyl amine **118** was treated with 13 mg of carbon tetrabromide and 10 mg of triphenyl-phosphine at room temperature. After 2 hr, the reaction mixture was concentrated, redissolved in 1 mL of anhydrous ethanol and treated with 20 mg of potassium thioacetate at room temperature. After reflux for 4 hr, the reaction mixture was diluted with ethyl acetate, washed with sat. sodium bicarbonate, water, brine, dried over $MgSO_4$, and concentrated. The crude product was purified by silica gel chromatography to afford the bis-acetylthioethyl amine. The amine was treated with NaOH in methanolic water at room temperature. After 12 h, the reaction mixture was extracted and with methylene chloride and the combined organic phases were dried over $MgSO_4$ and concentrated. The crude product was purified by silica gel chromatography to afford the disulfide **119** (6 mg, 0.009 mmol, 38% yield from **118**).

(x) Technetium Complex **120**

To a suspension of the disulfide **119** in 50% aqueous sulfuric acid is added ground sodium nitrite at 0°C, under which condition the Boc groups are removed prior to the tetrazotization. After 30 min, the reaction mixture is added salicylic acid dissolved in aq. sodium carbonate. Upon addition, the pH of the reaction mixture is adjusted to 9-10 by slow addition of precooled sat. sodium carbonate solution while keeping the reaction temperature under 10°C. After overnight at 4°C, the reaction mixture is acidified (pH 3) and the resulting precipitate is filtered with a Buchner funnel. The collected product mixture is purified with silica gel colum using DMF-hexane as an eluent system. The purified dye suspended in water is then treated with ammonium pertechnetate (NH_4TcO_4), sodium hydrosulfite, and the resulting suspension is refluxed for 5 hr. The reaction mixture is extracted with methylene chloride, dried over $MgSO_4$, and chromatographed to give the metal complex **120**.

SCHEME XI


NH2
COOH
Br
109
1) NaH
2) Boc2O
NHBoc
COOH
Br
110
1) ClOOCCH2CH3
2) NaBH4
NHBoc
OH
Br
111
AcCl
Swern Oxidation
NHBoc
CHO
Br
114
NHBoc
OAc
CHO
NHBoc
115
(Ph3P)4Pd
NHBoc
OAc
SnBu3
113
(Bu3Sn)2
(Ph3P)4Pd
NHBoc
OAc
Br
112
1) LiOH
2) CBr4/Ph3P
3) NaS
OCH3
NHBoc
S(p-MeO)Bn
CHO
NHBoc
116
1) NaBH4
2) CBr4/Ph3P
NHBoc
S(p-MeO)Bn
Br
NHBoc
117
HN(CH2CH2OH)2
NHBoc
S(p-MeO)Bn
OH
N
OH
NHBoc
118
1) CBr4/Ph3P
2) KSAc
3) NaOH/Air
NHBoc
S(p-MeO)Bn
S
S
N
NHBoc
119
1) NaNO2
2) salicylic acid
3) NH4TcO4
4) NaS2O4
HO
HOOC
N
N
O
Tc
N
S
S
S
L
N
N
OH
COOH
120

Example 21: Preparation of Tc Complex 122

(Prep. Scheme XII)

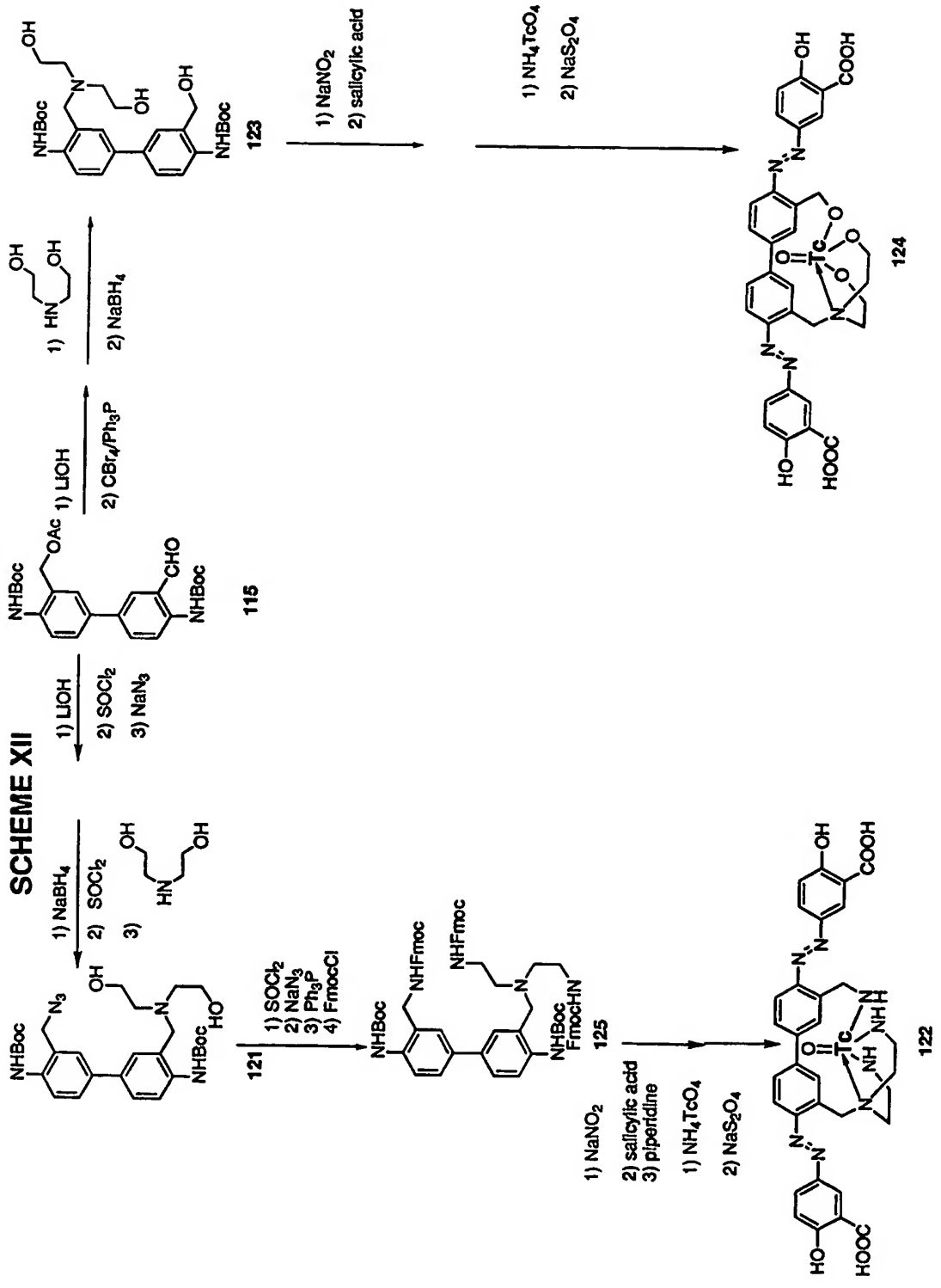
(i) Biphenyl Diol 121

To a solution of the benzoate 115 in THF and water is added LiOH at 0°C. After completion of the reaction, the product benzyl alcohol is treated with thionyl chloride at room temperature. The resulting benzyl chloride is subsequently reacted with sodium azide to give the biphenyl azide. A solution of the biphenyl azide in anhydrous MeOH is reduced with sodium borohydride to its azido alcohol. Upon reaction with thionyl chloride, the benzyl alcohol is converted to the biphenyl diol 121 via benzyl chloride.

(ii) Complex 122

The two hydroxy groups are converted into dibromide with either carbon tetrabromide/triphenyl phosphine system or bromine, which is then treated with excess sodium azide to give tri-azide. The tri-azide groups are reduced to the tri-amines with triphenyl phosphine, and subsequently protected with Fmoc group to afford the Fmoc protected amine. The Fmoc protected amine is tetrazotized with sodium nitrite in acid, under which condition the Boc groups are removed prior to the tetrazotization, to its tetrazonium salt. Treatment with 3 eq. salicylic acid affords the corresponding azo dye. Deprotection of Fmoc group with piperidine followed by Tc loading step using ammonium pertechtenate and sodium hydro sulfite in boiling water provides the desired metal complex 122.

SCHEME XII


1) NaBH4
2) SOCl2
3)
1) LiOH
2) SOCl2
3) NaN3
1) LiOH
2) CBr4/Ph3P
1) HN
2) NaBH4
1) NaNO2
2) salicylic acid
1) NH4TcO4
2) NaS2O4
1) SOCl2
2) NaN3
3) Ph3P
4) FmocCl
1) NaNO2
2) salicylic acid
3) piperidine
1) NH4TcO4
2) NaS2O4
121
115
123
124
125
122

Example 22: Preparation of Tc Complex **124**

(Prep. Scheme XII)

(i) Biphenyl Triol **123**

The benzoate **115** is hydrolyzed to its alcohol with LiOH, which is then treated with carbon tetrabromide and triphenyl phosphine to its bromide. The bromide is reacted with bis-ethanolamine and its aldehyde funtional group is reduced with sodium borohydride to the corresponding triol **123**.

(ii) Tc Complex **124**

The triol **123** is tetrazotized with sodium nitrite in acid under which condition the Boc groups are removed prior to the tetrazotization. Subsequent treatment with salicylic acid at pH around 9 provides the azo dye. The azo dye is treated with ammonium pertechtenate and sodium hydro sulfite in boiling water to give the complex **124**.

Example 23: Binding of NX_3 Technetium Complexes to β-Amyloid

This example illustrates the affinity of NX_3 technetium complexes for β1-40 amyloid fibrils. A similar protocol to that described in Example 6 is used.

Example 24: Synthesis of N_2S_2 Complexes (Prep. Schemes XIII and XIV)

(i) 2-Iodo-4,4'-Dinitrobiphenyl **77**

To a 250 mL 3-neck round-bottom flask charged with 7.8 g (30.16 mmol) of the amine **76** (see Case, F.H., J. Am. Chem. Soc. 68:2574-2577 (1946)) was added 50 mL of conc. sulfuric acid. After the mixture became homogeneous, it was cooled to 0°. In a separate 50 mL flask 2.8 g of sodium nitrite was slowly to 25 mL of sulfuric acid over 30 min. at 5°. The resultant solution was then slowly added to the amine solution with cooling. Manual agitation of the reaction mixture was necessary. Next, 75 mL of 85% phosphoric acid was added over one hour via addition funnel, during which time the temperature of the reaction mixture was kept below 10°. The mixture was agitated periodically to assure homogeneity. Conversion to the diazonium salt was monitored by adding a drop of the reaction mixture to 10 mL of water. When the solution turned light yellow and no insoluble material was formed, the reaction was complete. The reaction mixture was then allowed to warm to room temperature over 2 h and added to 500 mL of ice water with stirring. After

30 min, excess urea (6 g) was slowly added to destroy excess nitrous acid and the reaction mixture was stirred for 30 min. To this mixture was added an aqueous solution of potassium iodide (11.34 g in 75 mL). The reaction mixture was then heated to 70°. The reaction mixture was extracted with CH_2Cl_2, dried over $MgSO_4$ and concentrated, The crude product was purified by silica gel chromatography (1/1 Hexane/CH_2Cl_2) to afford the dinitroiodide product **77** 9.83 g, 26.86 mmol, 89% yield) as light yellow solid. ^1H NMR (DMSO-d_6) δ 8.75 (d, 1H, J = 2.1 Hz), 8.38-8.32 (m, 3H), 7.71-7.64 (m, 3H).

(ii) 2-Cyano-4,4'-Dinitrobiphenyl **78**

A solution of the iodide **77** (5.0 g, 13.66 mmol) and CuCN (1.65 g, 18.42 mmol) in 30 mL of anhydrous dimethylsulfoxide was heated (180°C) under an argon atmosphere. After 2 hr, the reaction mixture was added to a 0° solution of aqueous ammonium chloride (100 mL) and filtered through a Buchner funnel. The insoluble product was partitioned into methylene chloride and water. The separated organic layer was dried over $MgSO_4$ and concentrated. The crude product was purified by silica gel chromatography (1/1 Hexane/CH_2Cl_2 to 30%/69%/1% Hexane/CH_2Cl_2/MeOH) to afford the nitrile **78** (3.3 g, 12.27 mmol, 90% from **77**) as a light yellow solid. ^1H NMR (DMSO-d_6) δ 8.69 (d, 1H, J = 2.3), 8.56 (dd, 1H, J_1 = 8.6, J = 2.3), 8.44-8.41 (m, 2H), 7.80-7.76 (m, 3H).

(iii) Triamine **79**

A solution of nitrile **78** (750 mg, 2.79 mmol) in methanol (100 mL) was shaken with 10% Pd on C (200 mg) under H_2 (55 psi) for 24 h, using a Parr apparatus. The reaction mixture was filtered through Celite and concentrated to afford the diamino nitrile as a yellow oil (559 mg, 2.67 mmol, 96%) R_f = 0.54 (7:93 MeOH/CH_2Cl_2). The crude product was dissolved in tetrahydofuran (5 mL) and added in a dropwise manner to a suspension of $LiAlH_4$ (1 g, 26.4 mmol) in THF (30 mL) and the reaction mixture was heated to reflux under argon. After 17 h, the reaction mixture was cooled to room temperature and quenched by sequential addition of H_2O and 5% NaOH. The mixture was filtered to remove insoluble aluminum salts and the filtrate was extracted with CH_2Cl_2, dried over potassium carbonate, filtered, and concentrated in vacuo to give a triamine **79** as a tan oil (432 mg, 2.03 mmol, 76% from **78**). ^1H NMR (DMSO-d_6) δ 6.93 (d, J = 8.3, 2H), 6.77 (d, J = 8.5, 1H), 6.68 (d, J = 1.8, 1H), 6.55 (d, J = 8.4, 2H), 6.43 (dd, J = 8.4, 2.5, 1H), 4.98 (brs, 2H), 4.91 (brs, 2H), 3.32 (s, 2H), 3.17 (s, 2H).

(iv) Compound **81**

A solution of 2-aminoethanethiol•HCl **80** (11.4 g, 100 mmol), triphenylmethanol

(TrOH, 26.0 g, 100 mmol) in HCl (37%, 44.8 mL) and acetic acid (280 mL) was stirred for 5 h at 40 °C. The reaction mixture was concentrated in vacuo and the resulting white solid was washed with ether and dissolved in H_2O/CH_2Cl_2 (1:1). After adjusting the pH of the aqueous layer to ca. 14 with 1 N NaOH, the desired compound was removed by extraction. The combined organic layers were dried (K_2CO_3), filtered, and concentrated in vacuo to provide a white solid, S-(triphenylmethyl) 2-aminoethanethiol (28.8 g, 90.0 mmol, 90% from **80**). R_f = 0.50 (1:9 MeOH/CH_2Cl_2); ^{1}H NMR (DMSO-d_6) δ 7.35-7.20 (m, 15H), 2.43 (t, J = 6.1, 2H), 2.15 (t, J = 5.9, 2H). To a solution of S-(triphenylmethyl) 2-aminoethanethiol (3.00 g, 9.40 mmol) and triethylamine (951 mg, 9.40 mmol) in dry CH_2Cl_2 (30 mL) was added bromoacetyl bromide (1.90 g, 9.40 mmol) over 15-20 min at -20°. The reaction mixture was stirred at -20° for 30 min and at room temperature for 1 h. To this mixture was added an additional equivalent of S-(triphenylmethyl) 2-aminoethanethiol (3.00 g, 9.40 mmol) and triethylamine (951 mg, 9.40 mmol). After stirring for 16 h at room temperature, the reaction mixture was concentrated under reduced pressure. The crude product was purified by flash chromatography (1:99 MeOH/CH_2Cl_2) to give compound **81** as a white foam (4.64 g, 6.95 mmol, 67 % from amine **80**). R_f 0.60 (1:9 MeOH/CH_2Cl_2); ^{1}H NMR (DMSO-d_6) δ 7.77 (t, J = 6.1, 1H), 7.35-7.20 (m, 30H), 2.97 (dd, J = 6.0, 5.9, 2H), 2.86 (s, 2H), 2.35 (t, J = 6.1, 2H), 2.18 (m, 4H). See Photaki et al., J. Chem. Soc. (c) 2683-2687 (1970); Bryson, N.J., Neutral technetium (v) complexes with N,S-donor chelates. Ph.D. thesis, Massachusetts Institute of Technology, Cambridge, MA (1988).

(v) Compound **82**

To a solution of **81** (2.68 g, 3.96 mmol) in acetonitrile (100 mL) was added methyl 3-bromopropionate (2 mL, 28.8 mmol), $KHCO_3$ (500 mg), and K_2CO_3 (500 mg). The reaction mixture was refluxed under argon at 80°C for 12 h. After cooling to room temperature and filtering, the solution was concentrated. The crude product was purified by flash chromatography (hexane, then 1:3 ethyl acetate/hexane) to give compound **82** as a pale yellow oil (1.96 g, 2.58 mmol, 65% from **81**) R_f = 0.35 (1:3 EtOAc:Hexane) and recovered 0.67 g (1.0 mmol) of **81** (yield was 87 % based on recovered starting material). ^{1}H NMR (DMSO-d_6) δ 7.58 (t, J = 6.0, 1H), 7.31-7.22 (m, 30H), 3.47 (s, 3H), 2.94 (q, J = 6.5, 2H), 2.79 (s, 2H), 2.51-2.47 (m, 2H), 2.32-2.26 (m, 4H), 2.22-2.17 (m, 4H).

(vi) Compound **83**

To a solution of **82** (1.53 g, 2.0 mmol) in MeOH (15 mL), H_2O (10 mL), and THF (15 mL) was added LiOH•H_2O (168 mg, 4.0 mmol). The resulting solution was stirred for

3 h at room temperature. Organic solvent was removed under vacuum and the resultant aqueous solution was acidified to pH 4 with 10% HCl and extracted with ethyl acetate. The combined ethyl acetate fractions were dried over $MgSO_4$ and evaporated to give the acid as a white solid (1.50 g) (R_f = 0.71 (7:1:92 MeOH/acetic acid/CH_2Cl_2); ^1H NMR (DMSO-d_6) δ 7.67 (t, J = 5.8, 1H), 7.31-7.18 (m, 30H), 2.95 (q, J = 6.7, 2H), 2.79 (s, 2H), 2.51-2.46 (m, 2H), 2.30-2.16 (m, 8H). To a solution of the crude acid (1.50 g, 2 mmol) and N-hydroxysuccinimide (230 mg, 2.0 mmol) in THF (30 mL) was added 1,3-dicyclohexylcarbodiimide (430 mg, 2.0 mmol) in THF (10 mL). After stirring at room temperature for 2 h, the solution was filtered into a flask containing triamine **79** (405 mg, 1.9 mmol) in THF (10 mL). After 2 h at room temperature, the reaction mixture was concentrated and the crude product was purified by flash chromatography (CH_2Cl_2, then 3:97 MeOH/CH_2Cl_2) to give compound **83** as a brown solid (860 mg, 0.91 mmol, 45% from **82**). R_f = 0.49 (7:1:92 MeOH/acetic acid/CH_2Cl_2); ^1H NMR (DMSO-d_6) δ 8.09 (t, J = 5.0, 1H), 7.71 (t, J = 5.4, 1H), 7.29-7.19 (m, 30H), 6.89 (d, J = 8.4, 2H), 6.82 (d, J = 8.7, 1H), 6.55 (d, J = 8.3, 2H), 6.49-6.47 (m, 2H), 4.99 (brs, 2H), 4.95 (brs, 2H), 4.04 (d, J = 6.4, 2H), 2.95-2.91 (m, 2H), 2.76 (s, 2H), 2.51-2.49 (m, 2H), 2.35-2.31 (m, 2H), 2.22-2.13 (m, 6H).

(vii) Compound **84**

To a solution of **83** (50 mg, 0.053 mmol) in THF (6 mL), H_2O (3 mL), and 10% HCl (280 μL) at 5° was added a solution of $NaNO_2$ (8 mg, 0.12 mmol) in H_2O (40 μL). After stirring at 5° for 2 min, the resulting yellow solution was added dropwise to a solution of 4-amino-1-naphthalenesulfonic acid sodium salt (61 mg, 0.25 mmol), sodium acetate trihydrate (108 mg, 0.79 mmol), and Na_2CO_3 (10 mg, 0.094 mmol) in H_2O (1 mL) at 5°. A distinct color change from yellow to orange-red was immediately observed. After stirring at 5° for 3 min, THF (1 mL) was added. After 1 h at 5° the mixture was concentrated and the crude product was purified by flash chromatography (CH_2Cl_2, then 1:9, 3:7 MeOH/CH_2Cl_2) followed by preparative HPLC (0-5 min 10% MeOH, 5-20 min 10-100% MeOH gradient, 20-25 min 100% MeOH, R_v = 285-345 mL) to afford **84** as a red solid (32 mg, 0. 026 mmol, 42% from **83**). R_f = 0.29 (25:75 MeOH/CH_2Cl_2); UV(10 mM Na_2HPO_4, pH 7.4) λ_{max} 486 nm (ϵ = 4.36 x 10^4 $cm^{-1}\bullet M^{-1}$), 324 nm (ϵ = 4.56 x 10^4); ^1H NMR (CD_3OD) δ 8.81 (d, J = 8.1, 2H), 8.65 (s, 1H), 8.64 (s, 1H), 8.29 (d, J = 7.9, 1H), 8.28 (d, J = 7.8, 1H), 7.96 (d, J = 2.0, 1H), 7.91 (d, J = 8.1, 3H), 7.66 (t, J = 7.9, 2H), 7.53 (t, J = 8.4, 4H), 7.46 (d, J = 7.5, 1H), 7.30-7.25 (m, 12H), 7.16-7.07 (m, 18H),

4.48 (s, 2H), 2.97 (t, J = 6.8, 2H), 2.86 (s, 2H), 2.62 (t, J = 5.9, 2H), 2.34-2.25 (m, 8H). See Ashburn et al., Chemistry and Biology 3:351-358 (1996); Han et al., J. Am. Chem. Soc. 118:4506-4507 (1996).

(viii) Compound **86**

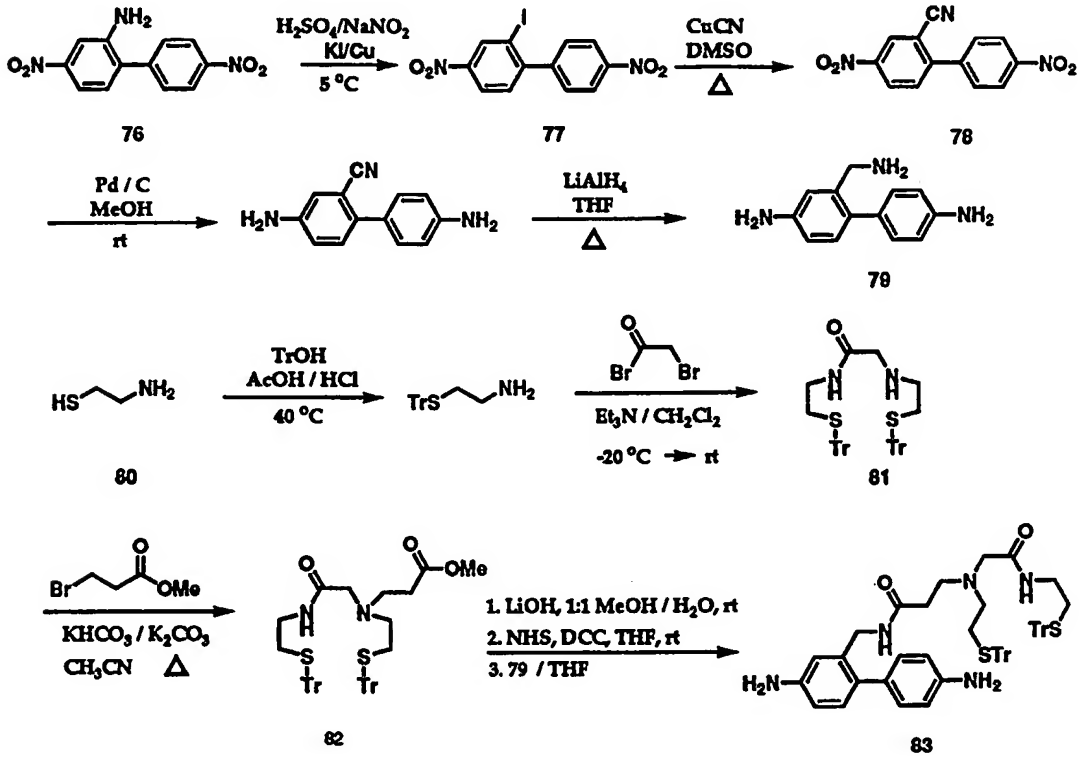
To a solution of **83** (20 mg, 0.02 mmol) in THF (3 mL), H_2O (1.5 mL), and 10% HCl (80 μL) at 5°C was added $NaNO_2$ (4 mg, 0.06 mmol) in H_2O (20 μL). After stirring at 5° for 2 min, the resulting yellow solution was added dropwise a solution of 250 uL 1-hydroxy-2-naphthoic acid (1:1 THF/0.5 M Na_2CO_3) at 5°. A distinct color change from yellow to orange-red was immediately observed. After stirring at 5° for 1 h, the solution was warmed to room temperature and acidified to pH 4-5 by addition of 10% HCl. This solution was extracted by EtOAc and dried over Na_2SO_4. Purification by flash chromatography (CH_2Cl_2, then 1:9, 3:7 MeOH/CH_2Cl_2) followed by preparative HPLC (0-5 min 10% MeOH, 5-20 min 10-100% MeOH gradient, 19-21 min 100% MeOH, R_v = 285-315 mL) afforded **86** as a golden yellow solid (6 mg, 4.5 umol, 25% from **83**). R_f = 0.35 (25:75 MeOH/CH_2Cl_2); ^{1}H NMR (CD_3OD) δ 8.90 (dd, J = 8.1, 7.8, 2H), 8.56 (s, 2H), 8.40 (d, J = 8.1, 2H), 8.07-7.99 (m, 4H), 7.66-7.50 (m, 7H), 7.29-7.22 (m, 12H), 7.14-7.03 (m, 18H), 4.50 (s, 2H), 2.91 (t, J = 6.9, 2H), 2.82 (s, 2H), 2.64 (m, 4H), 2.32-2.19 (m, 6H). See Ashburn et al., Chemistry and Biology 3:351-358 (1996); Han et al., J. Am. Chem. Soc. 118:4506-4507 (1996).

(ix) Compound **85**

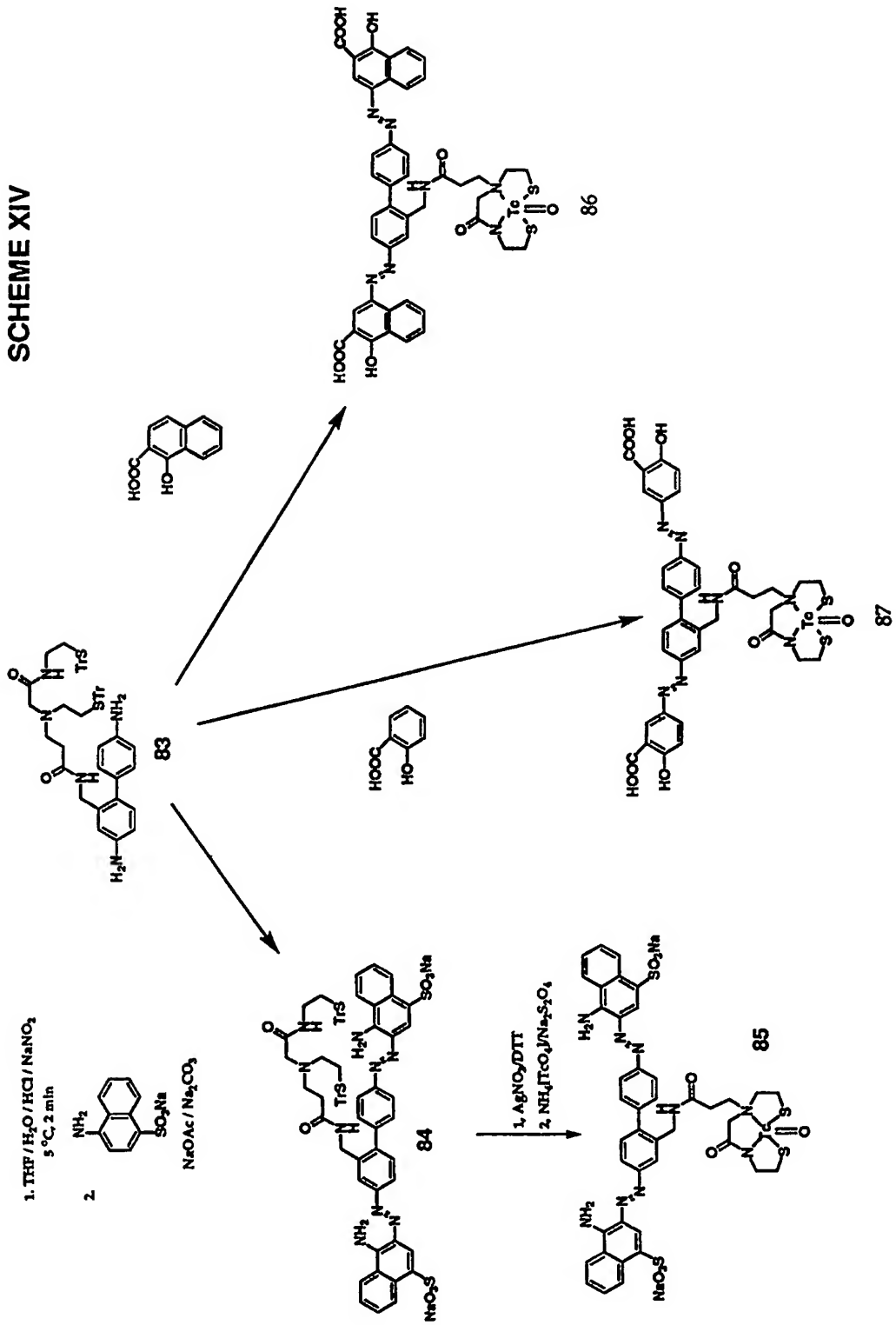
To a solution of **84** (9.0 mg, 6.4 μmol) in MeOH/H_2O (1:1, 3 mL) was added 0.1 M aqueous silver nitrate (330 μL). The dark silver mercaptide derived from **84** precipitated mmediately. After 5 min, the precipitate was collected by centrifugation and resuspended in THF/H_2O (1:1, 3 mL). The solution was treated with 0.1 M dithiothreitol (DTT, 660 μL) for 5 min followed by 10% Na_2CO_3 (80 μL) and the supernatant was collected by centrifugation. To a 10 mL vial containing 25 mM $NH_4[TcO_4]$ (600 μL, New England Nuclear, Boston, MA) and 0.01 N NaOH (4.5 mL, pH 12) was added sequentially the supernatant and 1 M $Na_2S_2O_4$ (30 μL) in 0.01 N NaOH at room temperature. The reaction mixture was heated at ca. 75°C for 30 min, cooled to room temperature, and purified by flash chromatography using C18 corasil (37-50 μm, Waters, Milford, MA, 2 x 4 cm) (washed with H_2O, then eluted with MeOH). Purification by preparative HPLC (0-5 min 0% MeOH, 5-10 min 10% MeOH, 10-25 min 10-100% MeOH gradient, R_v = 285-345 mL) afforded a red solid **85** (7.4 mg, 7.1 μmol, 88% from **84**). Specific activity = 1.26

mCi/mmol; UV (10 mM Na_2HPO_4, pH 7.4) λ_{max} 482 nm (ϵ = 1.19 x 10^4 $cm^{-1} \bullet M^{-1}$), 328 nm (ϵ = 1.43 x 10^4); ^1H NMR (CD_3OD) δ 8.77 (d, J = 9.1, 2H), 8.62 (s, 1H), 8.60 (s, 1H), 8.30 (d, J = 7.7, 2H), 8.02-7.90 (m, 4H), 7.67-7.47 (m, 7H), 4.56-4.48 (m, 4H), 3.54-3.47 (m, 2H), 3.13-2.40 (m, 10H); IR 1651, 1574, 1416, 1338, 1180, 1047, 960 (Tc = O) cm^{-1}. MALDI MS for $C_{42}H_{41}N_9O_9S_4Tc$ $[M]^+$, calcd 1041, found 1042. See Han et al., J. Chem. Soc. 118:4506-4507 (1996).

SCHEME XIII



SCHEME XIV


1. THF / H2O / HCl / NaNO2
5 °C, 2 min
2
NaOAc / Na2CO3
83
84
85
86
87
1. AgNO3/DTT
2. NH4TcO4/Na2S2O4

Example 25: Binding of N_2S_2 Technetium Complexes to β-Amyloid

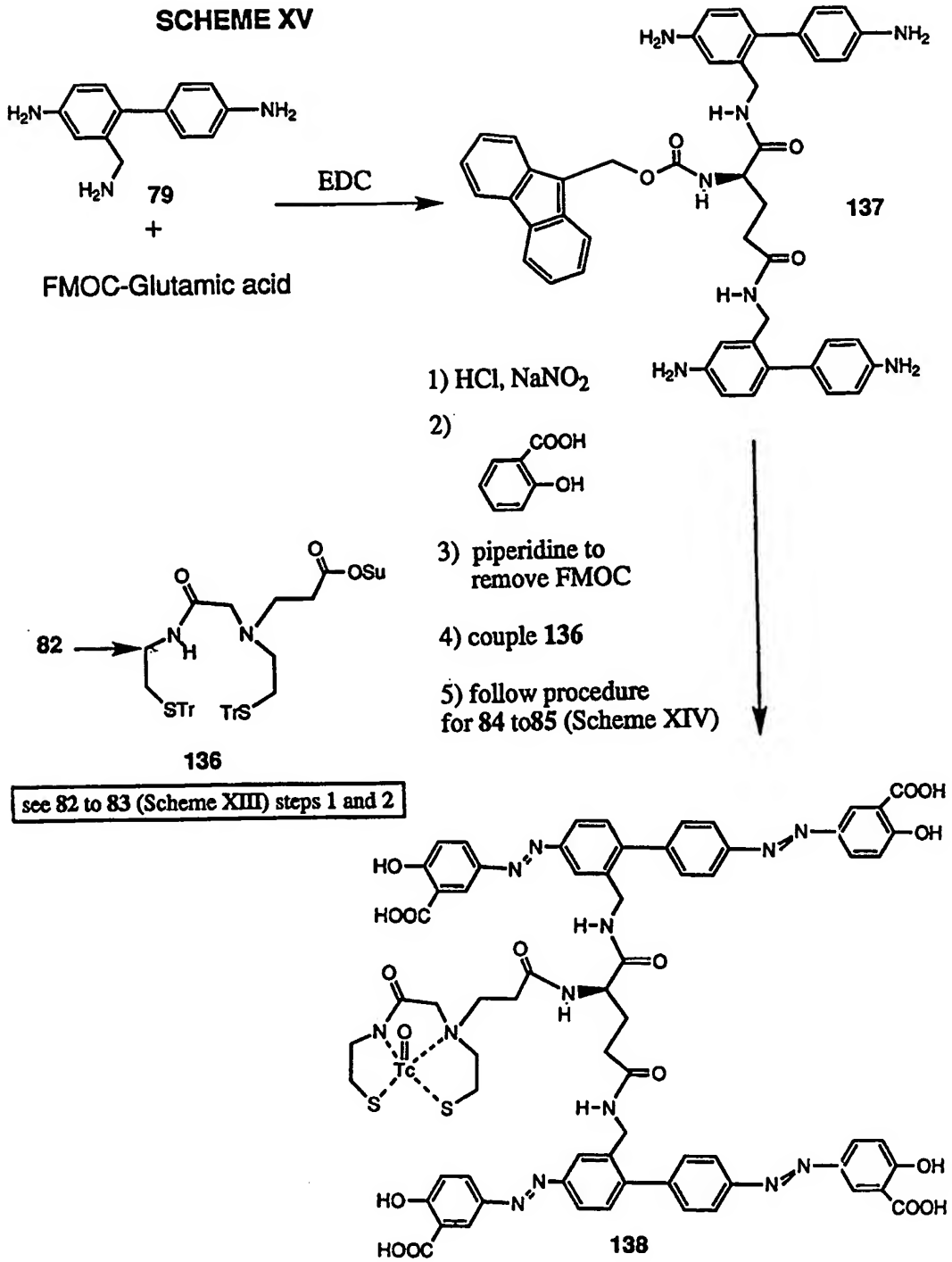
This example illustates the affinity of N_2S_2 technetium complexes for β1-40 amyloid fibrils. A similar protocol to that described in Example 6 is used.

Example 26: Preparation of Dimeric Amyloid Probes

(a) Preparation of N_2S_2 Chyrsamine G-dimer 138 (Prep. Scheme XV)

To a solution of FMOCGlu in THF (ca. 0.2 M) is added 2 equiv. of 1,3,dicycohexylcarbodiimide and 2 equiv. of N-hydroxy succinimide. After several hours, the solution is concentrated and the crude bis-NHS ester is precipated as a white solid by addition of ether. The crude solid is dissolved in 1:1 THF:0.25 M Na_2CO_3 and 1 equiv. of triamine **79** is added. The solution is stirred at room temperature and extracted with dichloromethane. The combined organic phases are dried over sodium sulfate and concentrated to provide, after purification, the FMOC-protected tetraamine **137**. **137** is diazotized and coupled to ortho salicylic acid as detailed in Example 24. The product is deprotected with piperidine and coupled to the N-hydroxysuccinimide ester **136** derived from **82**. Technetium-99m is loaded according to Example 24, to afford compound **138**.

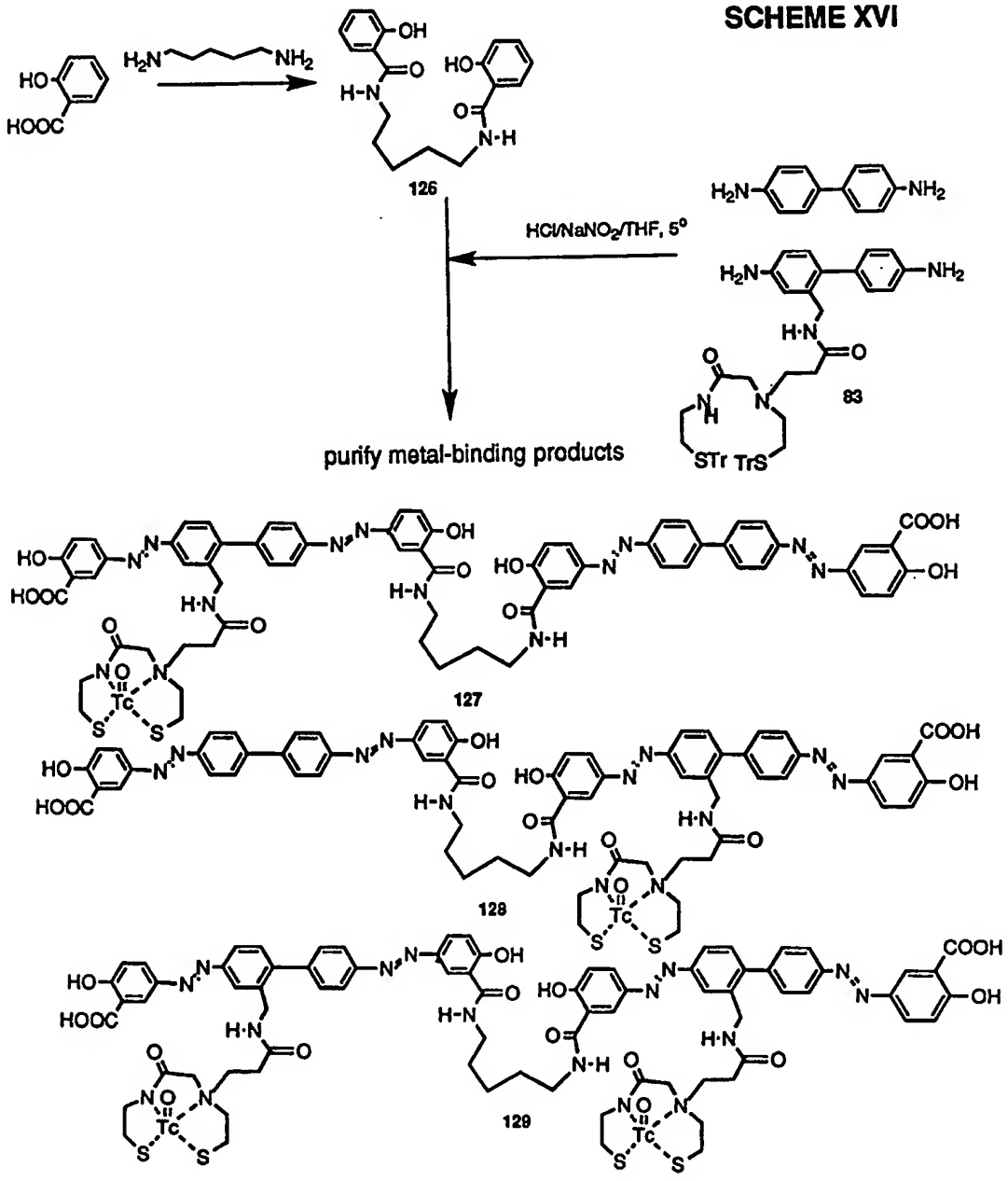
SCHEME XV


79
+
FMOC-Glutamic acid
EDC
137
1) HCl, NaNO2
2)
3) piperidine to remove FMOC
4) couple 136
5) follow procedure for 84 to85 (Scheme XIV)
82
136
see 82 to 83 (Scheme XIII) steps 1 and 2
138

(b) Preparation of Head-to-Tail Dimer 127 (Prep. Scheme XVI)

Ortho salicylic acid is treated with oxalyl chloride (1 equiv.) in methylene chloride to afford the acid chloride, which is then added to a solution of 1,5 diamino pentane (0.5 equiv.) to afford, after concentration and purification, compound **126**. **126** is added to a mixture of two bisdiazonium salts, derived from **83** and 4,4' biphenyl diamine by the procedure detailed in Example 24. The four possible coupling products are isolated and separated; the three shown in Scheme XVI, **127**, **128** and **129**, are possible amyloid probes.

SCHEME XVI



Example 27: Binding of N_2S_2 Technetium Dimers to β-Amyloid

This example illustrates the affinity of N_2S_2 technetium dimers for β1-40 amyloid fibrils. A similar protocol to that described in Example 6 is used.

Example 28: Preparation of Amyloid Binding Fluoresent Ligands (Prep. Scheme XVII)

(a) Congo Red Fluorescein Ligand 131

Triamine **79** (10 mg, 0.045 mmol) was dissolved in 1.0 mL of (1:1) THF/0.25 M Na_2CO_3 and Fluorescein succinimidyl ester (22 mg, 0.045 mmol) in 1 mL of (1:1) THF/0.25 M Na_2CO_3 was added. After stirring overnight, the solvent was evaporated and column chromatography on silica gel (0-25% MeOH/CH_2CL_2) to give the Fluorescein amine coupled compound **130** (20 mg, 75%). ^1H NMR (CD_3OD) δ 8.45 (s, 1H), 8.10 (d, J = 8.1, 1H), 7.28 (d, J = 8.1, 1H), 7.05 (d, J = 9.0, 1H), 6.52-7.06 (m, 12H), 4.52 (s, 2H), 1.95 (s, 1H). Congo Red with Fluorescein ligand was prepared by dissolving the Fluorescein diamine **130** in THF, H_2O and 10% HCl at 5°C, followed by addition of $NaNO_2$ in H_2O. After stirring at 5°C for 2 min, the resulting yellow solution was added dropwise a solution of 4-amino-1-naphthalenesulfonic acid sodium salt, sodium acetate trihydrate and Na_2CO_3 at 5°C. This dimer **131** was purified by flash column chromatography on silica gel (0-25% MeOH/$CH_2Cl_2CH_2CL_2$) to give a red oil.

(b) Congo Red Rhodamine Ligand 133

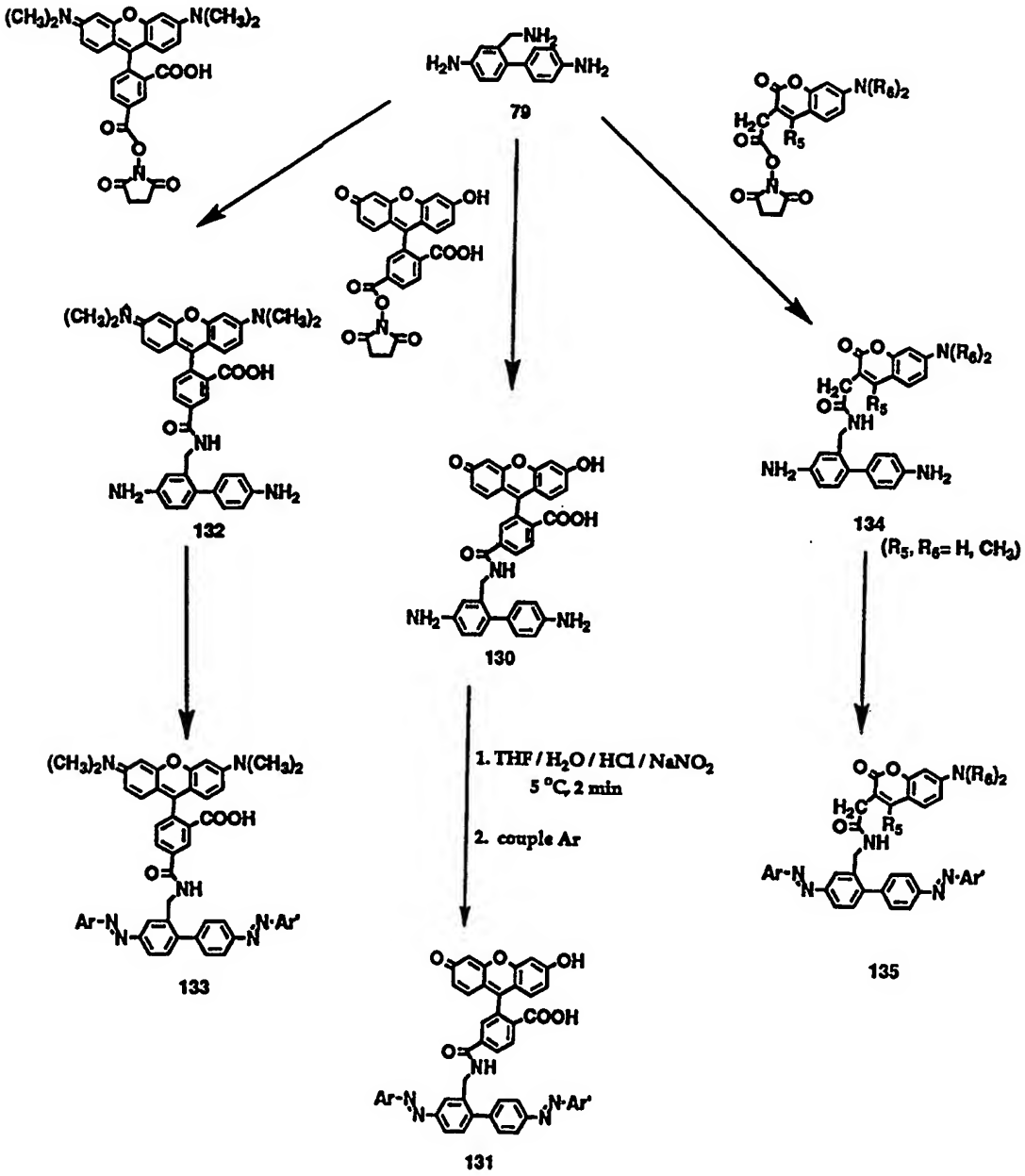
Triamine **79** is dissolved in 1 mL of (1:1) THF/0.25 M Na_2CO_3 and the Fluorescin succinimidyl ester in 1 mL of (1:1) THF/0.25 M Na_2CO_3 is added. After stirring overnight, the solvent is evaporated and column chromatography on silica gel (0-25% MeOH/ $CH_2Cl_2CH_2CL_2$) to give the Rhodamine amine coupled compound **132**. Congo Red with Rhodamine probe is prepared by dissolving the Rhodamine diamine **132** in THF, H_2O and 10% HCl at 5°C, followed by addition of $NaNO_2$ in H_2O. After stirring at 5°C for 2 min, the resulting solution is added dropwise a solution of 4-amino-1-naphthalenesulfonic acid sodium salt, sodium acetate trihydrate and Na_2CO_3 at 5°C. This dimer **133** is purified by flash column chromatography on silica gel (0-25% MeOH/$CH_2Cl_2CH_2CL_2$).

(c) Congo Red Coumarin Ligand 135

Triamine **79** is dissolved in 1 mL of (1:1) THF/0.25 M Na_2CO_3 and the Coumarin Succinimidyl ester in 1 mL of (1:1) THF/0.25 M Na_2CO_3 is added. After stirring overnight, the solvent is evaporated and column chromatography on silica gel (0-25%

$MeOH/CH_2Cl_2CH_2CL_2$) to give the Coumarin amine coupled compound **134**. Congo Red with Coumarin ligand is prepared by dissolving the Coumarin diamine **134** in THF, H_2O and 10% HCl at 5°C, followed by addition of $NaNO_2$ in H_2O. After stirring at 5°C for 2 min, the resulting solution is added dropwise a solution of 4-amino-1-naphthalenesulfonic acid sodium salt, sodium acetate trihydrate and Na_2CO_3 at 5°C. This dimer **135** is purified by flash column chromatography on silica gel (0-25% $MeOH/CH_2Cl_2CH_2CL_2$).

SCHEME XVII



Example 29: Synthesis of Dimeric Fluorescent Ligands

Amine **79** is coupled to the appropriate fluorescent chromophore (FL, general formula), activated as the N-hydroxy succinimide ester (available from Pierce, Rockville, IL). The resultant compound (see Scheme XVII) is diazotized (HCl, $NaSO_2$, THF, 5°) according to the procedure used for the preparation of the N_2S_2 dimer, and coupled to two equivalents of the appropriate aromatic (Ar) compound, to provide fluorescent amyloid ligands. For example, by substituting fluorescent diamines **130**, **132** or **134** (Scheme XVII) for diamine **83** in Scheme XVI, a series of head-to-tail fluorescent dimers can be assessed. Also, by substituting the commerically available fluorophore-NHS esters (Pierce) for compound NHS ester **82** in Scheme XV, another class of fluorescent dimers can be produced.

Example 30: High-Throughput Fluorescent Assay for Inhibitors of Intracellular β-Amyloid Aggregation

A cell line which produces intracellular β-amyloid is used. See, e.g., Martin et al., J. Biol. Chem. 270:26727-26730 (1995). Other cell lines also can be used, e.g., Down's syndrome human neurons isolated from Down's syndrome fetuses, or guinea pig neurons which have been treated with hydrogen peroxide. The cells are plated into a 96-well format. Solutions of candidate inhibitors are added and the cultures are incubated for between thirty minutes and 24 hours. At this time, the medium is removed by filtration and the cells are permeabilized using standard immunostaining methods. A solution of the fluorescent probe is added. After one to thirty minutes, the cells are washed (by filtration) several times to remove free probe. The plates are then analyzed in order to determine which wells retain the fluorescent probe. The wells which do NOT retain fluorescence contain a potential inhibitory compound or compounds. That is, an inhibitor inhibits the formation of β-amyloid aggregate and, therefore, the fluorescence signal. Those compounds which inhibit intracellular aggregation in this screen are further analyzed using standard fluorescence microscopy procedures.

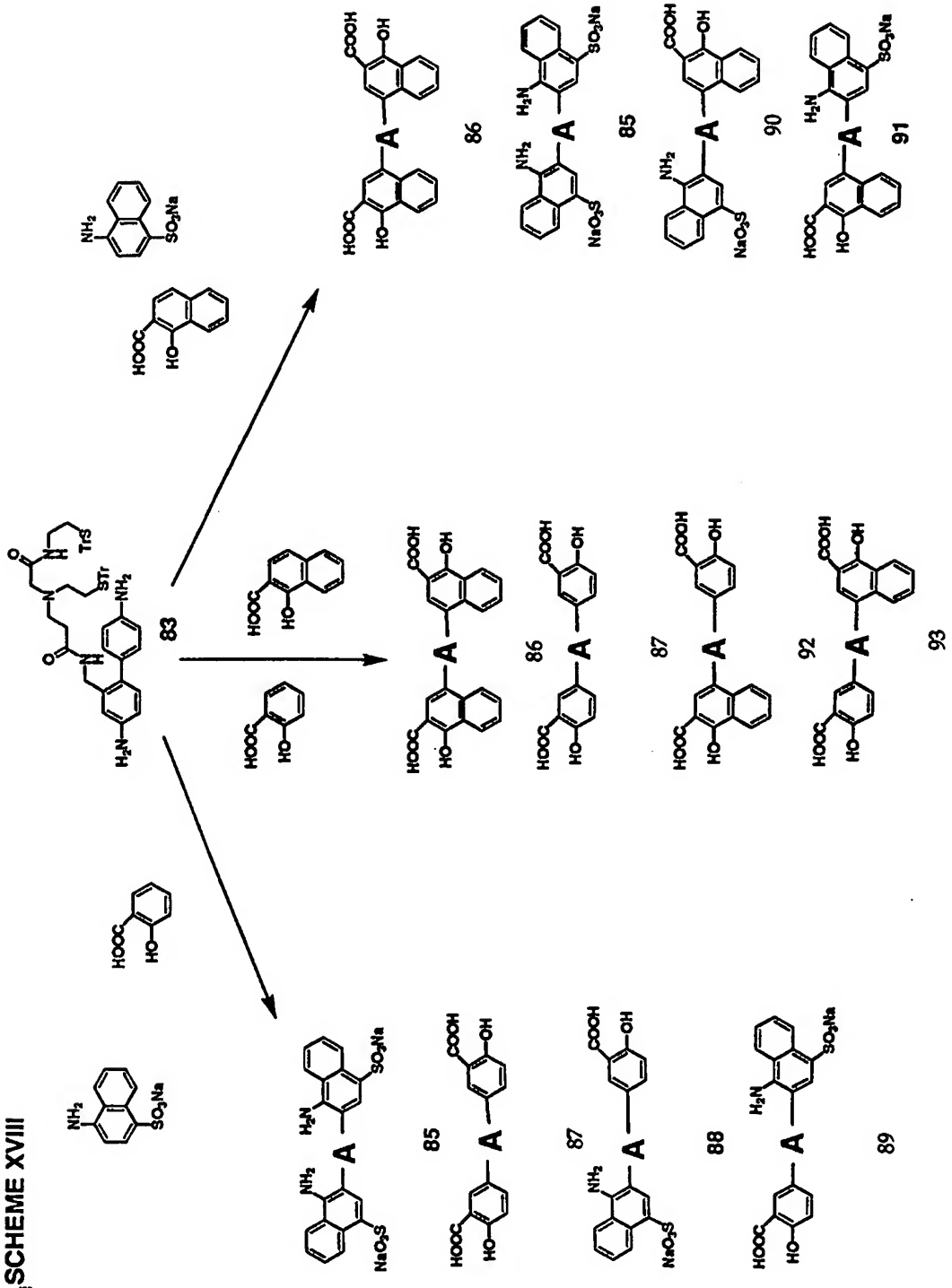
Example 31: Combinatorial Synthesis of Labeled Compound Libraries

(a) Labeled N_2S_2 Libraries (Prep. Scheme XVIII)

Scheme XIV illustrates the synthesis of a library of three molecules (**85**, **86** and **87**) in which Ar = Ar'. These compounds are individually tested as described in Examples 33, 34 or 35. Libraries of greater complexity are synthesized and screened using the same principles.

Using any three aromatic groups described above as Ar or Ar', six compounds are possible in which Ar and Ar' are different. These are made by exposing the bis-diazonium salt derived from **83** to mixtures of two aromatic compounds (Scheme XVIII). The two aromatic compounds are not present in equimolar amounts, but rather are in a ratio determined by their relative reactivity, such that all four possible products are produced in equimolar ratios (see Scheme XVIII). This mixture is screened as described in Examples 33, 34 or 35, to determine the relative affinities for particular amyloid aggregates. Alternatively, the bis-diazonium salt derived from **83** is exposed to a mixture of all three aromatic compounds, to generate all nine possible compounds (**85-93**), which are screened for binding. Because it is difficult to generate approximately equimolar amounts of all possible adducts from more complex mixtures, larger libraries are generated from 2 or 3 aromatic compounds to produce groups of 4 or 9 compounds per well.

SCHEME XVIII



(b) Labeled N_2S_2 Dimer Libraries

Triamine **79** is protected with an acid-stable protecting group such as the fluorenylmethoxy carbonyl (FMOC) group, using standard procedures. The protected triamine is diazotized (dissolved in THF/water at 5°C and diazotized by treatment with hydrochloric acid and sodium nitrite, as described in Example 24(vii)), and coupled to mixtures of aromatics as described above for the diamine **83**. Using six aromatics, 36 FMOC-protected compounds are generated in groups of four. These compounds are deprotected by standard methods (e.g., piperidine, methylene chloride), and coupled to bis-N-hydroxysuccinimide esters that contain a protected (FMOC or tBOC) or derivatized (with N_2S_2 precursor) amino group. Thus, a group of four bis aromatic adducts (in approximately equimolar ratio) are coupled with a single diester to produce up to 16 compounds (if the diester is symmetric, there will be fewer adducts) in approximately equimolar ratio. Amyloid binding compounds are identified by any of the screening methods described in Examples 33-35. Libraries having, e.g., 12,960 compounds (36x36x10), are easily generated and screened by this procedure.

Example 32: Generation and Isolation of β-Amyloid Protofibrils

This example illustrates a method for generating and isolating protofibrils using synthetic Aβ peptides. A stock solution was made by dissolving Aβ1-40 in DMSO to a final concentration of 2 mM and filtering through a 0.2 μm filter to remove undissolved material. An aliquot of the DMSO stock solution (10μL) was diluted into 90 μl aqueous pH 7.4 buffer (10 mM phosphate, 100mM NaCl) and thoroughly mixed. This solution was incubated at room temperature without agitation and protofibrils with lengths often exceeding 175 nm were visible by the second day of incubation. Protofibrils of similar lengths were also formed after similarly prepared incubations of Aβ1-42 at 20 μM were incubated for 8 days. Incubations of these peptides at lower concentrations generated protofibrils more slowly.

Protofibrils are isolated by sequential filtration of the early aggregation mixture through appropriate pore size and MW cutoff membranes to separate protofibrils from fibrillar and monomeric Aβ based on size differences. Aliquots of aggregation mixtures containing protofibrils are first filtered through an 0.2 μm pore size membrane to retain fibrils and allow Aβ protofibrils and monomers to pass into the filtrate. The peptide concentration of this filtrate is determined by quantitative amino acid analysis and aliquots of

this filtrate are then centrifuged through 0.2 μm cutoff filter to separate protofibrils in the retentate from monomeric Aβ in the filtrate. The amount of monomeric Aβ remaining in the filtrate is determined by quatitative amino acid analysis and subtraction of this value from the peptide concentration of the first filtrate provides the amount of Aβ retained above the membrane in the form of protofibrils. The retained protofibrils are then resuspended in an appropriate aqueous buffer for use.

Example 33: Screening Test Compounds For β-Amyloid Binding Ligands

This example illustrates treatment of various types of β-amyloid with test compounds to screen for compounds which are able to bind to the various types of β-amyloid. β-amyloid is obtained by standard methods from naturally-derived neuritic plaque (mostly fibrillar by electron miscroscopy), naturally-derived diffuse amyloid (not fibrillar by electron microscopy; Congo Red negative), synthetic Aβ1-40 protofibrils, synthetic Aβ1-42 protofibrils, synthetic Aβ1-40 type-1 fibrils, synthetic Aβ1-40 type-2 fibrils, synthetic Aβ1-42 type-1 fibrils or synthetic Aβ1-42 type-2 fibrils. Compounds which bind strongly to any or all of the above can be used as imaging ligands or amyloid aggregation inhibiting agents.

Stock solutions of the test compounds are prepared in aqueous buffer solutions and the concentrations are determined by calculation from the maximum absorbance in the UV-VIS spectra. Protofibrils are isolated as described in Example 32. Type-1 and type-2 fibrils are isolated as a mixture by filtration through a semipermeable filter which retains the fibrils. Neuritic plaque is isolated as described in Roher et al., J. Biol. Chem. 268:3072-3083 (1993), and diffuse amyloid is isolated as described in Gowing et al., J. Biol. Chem. 269:10987-10994 (1994). The isolated β-amyloid is resuspended in buffer containing the test compound, incubated for 30 min. at 25°C and centrifuged through a 0.2 μm cutoff membrane. The concentration of free ligand for use in Scatchard calculations is determined by calculation from the maximum absorbance in UV-VIS spectra taken of the filtrate. When using a radioactive compound, the amount of free ligand is determined by measuring the radioactivity of the filtrate. In this way, an effective binding constant is determined.

A similar protocol can be used to screen test compounds which bind to other amyloid proteins, e.g., Islet amyloid polypeptide, Ig light chain, transthyretin, lysozyme or β_2-microglobulin.

Example 34: Screening for Compounds Which Bind Specifically to β-Amyloid Protofibrils But Not to Other β-Amyloid Fibrils

This example illustrates treatment of various types of β-amyloid fibrils with test compounds to screen for compounds which are able to bind specifically to β-amyloid protofibrils but not to other types of β-amyloid fibrils. Compounds which bind strongly to the protofibrils can be used as imaging ligands or amyloid aggregation inhibiting agents.

The test compounds are individual compounds or libraries of compounds. Stock solutions of the test compounds are prepared in aqueous buffer solutions and the concentrations are determined by calculation from the maximum absorbance in the UV-VIS spectra. Protofibrils are isolated as described in Example 32. Type-1 and type-2 fibrils are isolated as a mixture by filtration through a semipermeable filter which retains the fibrils. Neuritic plaque is isolated as described in Roher et al., J. Biol. Chem. 268:3072-3083 (1993), and diffuse amyloid is isolated as described in Gowing et al., J. Biol. Chem. 269:10987-10994 (1994). The isolated β-amyloid is resuspended in buffer containing the test compound, incubated for 30 min. at 25°C and centrifuged through a 0.2 μm cutoff membrane. The concentration of free ligand for use in Scatchard calculations is determined by calculation from the maximum absorbance in UV-VIS spectra taken of the filtrate. When using a radioactive compound, the amount of free ligand is determined by measuring the radioactivity of the filtrate. For a library of compounds, tested at equal concentrations, strong binders are defined as those for which the amount of free ligand is lowest.

Each screen involves the incubation of the solution of the test compound(s) with the insoluble aggregate of one of the above described types of β-amyloid. The mixture is then filtered through a filter which has been previously determined to retain the β-amyloid aggregate. The desired test compound(s) are retained by the β-amyloid aggregate. By running two or more screens sequentially, compound(s) are identified which bind selectively to one form of β-amyloid aggregate.

For example, a library of compounds obtained as described in Example 31, is incubated with type-1 or type-2 fibrils, and then filtered. Library synthesis affords one compound each in every well of a 96-well plate. Each well has a 0.2μm filter at the bottom. Fibrils are added to each well and, after a 30 minute incubation, the mixtures are filtered and the amount of compound in the filtrate is measured. Those members of the original group of compounds which are not retained by the filter, are subjected to a second screen with protofibrils. Compounds which are retained by the protofibrils on the filter are specific

binders for protofibrils. These compounds are identified by their position in the 96-well plate: the position encodes the subunits added during the synthesis. (See Example 31).

Example 35: Identification of Amyloid-Binding Compounds in Combinatorial Libraries Using a Competitive Binding Assay

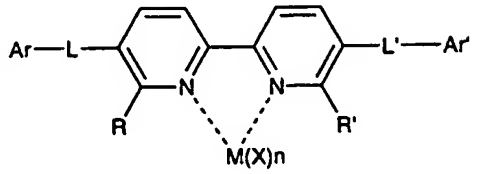
This example illustrates the screening of the combinatorial libraries constructed in Example 31, for amyloid binding compounds, e.g., to protofibrils, type-1 fibrils, type-2 fibrils, neuritic plaque and/or diffuse amyloid, comprised of, e.g., Aβ1-40 or Aβ1-42. In each case, the amyloid aggregate is added to one of, e.g., 96 wells containing an aqueous solution (pH 7.4, 10 mM phophate buffer) of a pure compound or a defined mixture of compounds from the combinatorial synthetic library. After a thirty minute incubation period, the mixture is filtered into a filtrate well through a filter which retains amyloid aggregate and bound compound(s). The UV/vis spectrum and/or the HPLC profile of the filtrate from each well is compared to the profile before exposure to the amyloid aggregates and subsequent filtration. Filtrate wells in which the composition, either in relative or absolute terms, is significantly different from that of the original well is further deconvoluted. Deconvolution consists of the separate synthesis and evaluation for amyloid binding (see Example 33) of each compound contained in the original mixture. In this way, those compounds in a synthetic combinatorial library with a relatively high affinity for a given amyloid aggregate are rapidly identified.

By placing two or more filtration steps in sequence, compounds which are selective in their binding properties can be identified. For example, addition of type-1 fibrils to the original wells, followed by filtration, will produce a filtrate library which contains no compounds with high affinity for type-1 fibrils. This library is treated with amyloid protofibrils and subjected to a second filtration step. By comparison of the filtrates from the first step to those from the second, compounds which bind the protofibrils are identified as described above. These compounds do not have high affinity for the type-1 fibrils.

Those skilled in the art will be able to ascertain, using no more than routine experimentation, many equivalents of the specific embodiments of the invention described herein. These and all other equivalents are intended to be encompassed by the following claims.

CLAIMS

1. An amyloid binding compound of the formula



(I)

and pharmaceutically acceptable salts thereof,

wherein

R and R^1 are H, N_2H_x (x is 0, 1, 2, 3 or 4), CH_2OH, CH_2NH_2, CH_2SH, o-$C_6H_4CH_2COOH$, $CH_2NHCH_2CH_2SH$, $CH_2P(CH_3)_2$, or $CH_2PCH_2CH_2P(CH_3)_2$, and can be the same or different from each other, and if R or R^1 is not H it can additionally bind or not bind to M, and if R or R^1 is H it cannot bind to M;

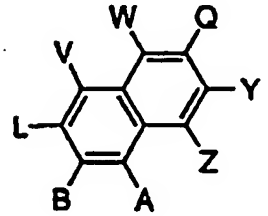
M is ^{99m}Tc, ^{111}In, ^{90}Y, ^{99}Tc, ^{186}Re, Cd, Zn, Co, Cu, Fe, Ni, or oxo forms of these metals;

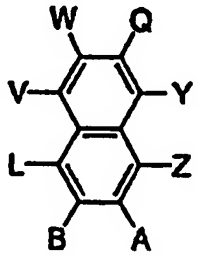
X is Cl, I, Br, F, $P(R^2)_3$ (R^2 is C_{1-6} hydrocarbon), $P(Ar^2)_3$ (Ar^2 is aryl or substituted aryl), R^3NC (R^3 is C_{1-6} hydrocarbon), Ar^3NC (Ar^3 is aryl or substituted aryl), SR^4 (R^4 is CH_2CH_2SH or C_{1-6} hydrocarbon), or $P(R^5)_2R^6$ (R^5 is C_{1-6} hydrocarbon; R^6 is C_{1-6} hydrocarbon or $CH_2CH_2P(CH_3)_2$, and each X can be the same or different from each other;

n is the number 1, 2, 3 or 4;

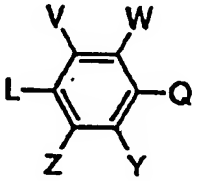
L and L' are -N=N-, -CONH-, -NHCO-, -HN-NH-, or -C=C-, and can be the same or different from each other; and

Ar and Ar' are



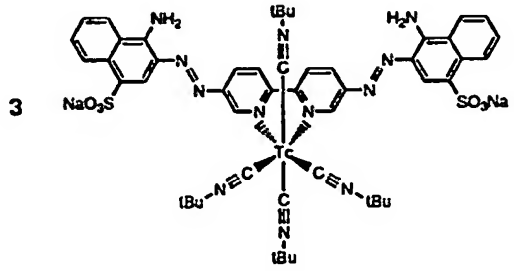


or



and can be the same or different from each other, where each of V, W, Q, Y, Z, A and B is OH, COOH, H, R^5 (R^5 is C_{1-6} hydrocarbon), CO_2R^6 (R^6 is C_{1-6} hydrocarbon), $CONH_2$, CN, NH_2, CH_2NH_2 or SO_3 and can be the same or different from each other.

2. A compound according to claim 1 wherein the formula is selected from the group consisting of:



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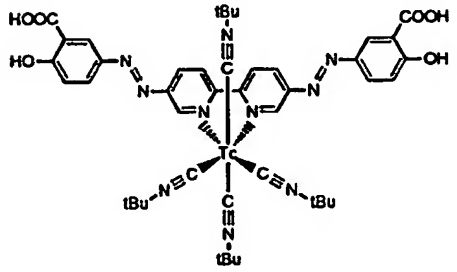
and



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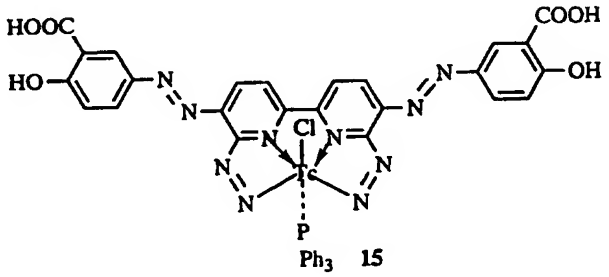
3. A compound according to claim 1 wherein the formula is selected from the group consisting of:



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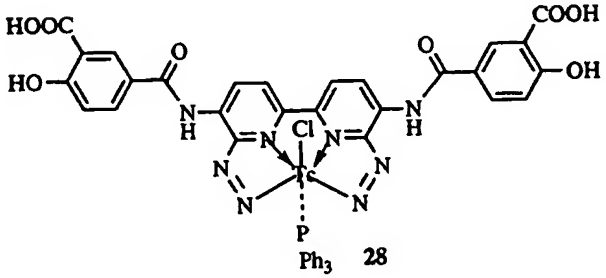

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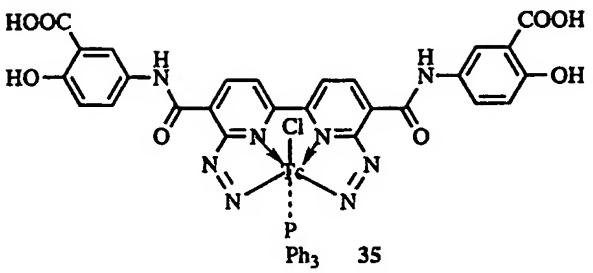

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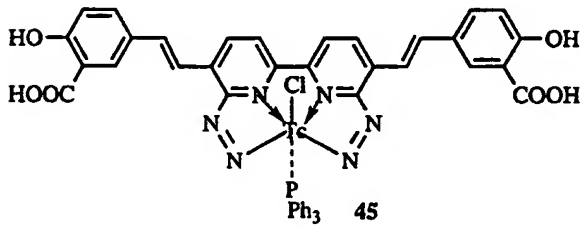

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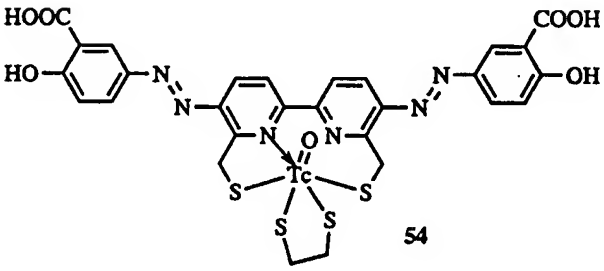

and

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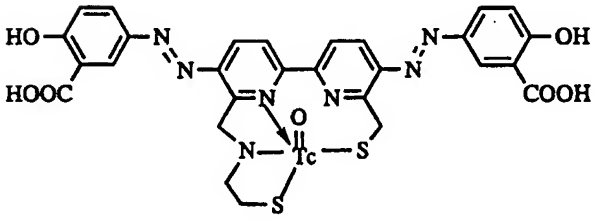
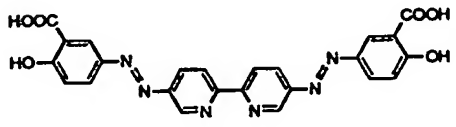
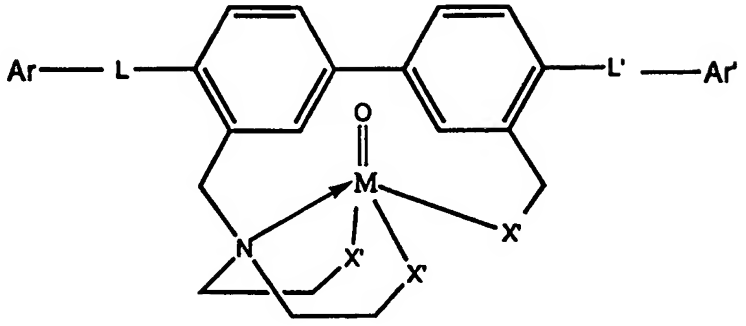


66

4. An amyloid binding compound of the formula



5. An amyloid binding compound of the formula



(II)

and pharmaceutically acceptable salts thereof,

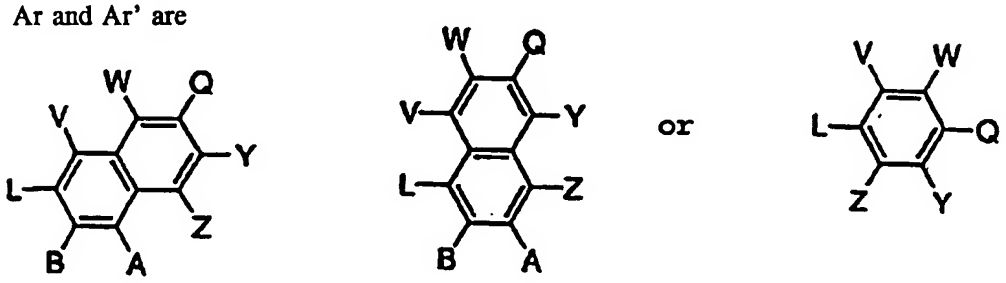
wherein

M is ^{99m}Tc, ^{111}In, ^{90}Y, ^{99}Tc or ^{186}Re;

X' is S, NH or O;

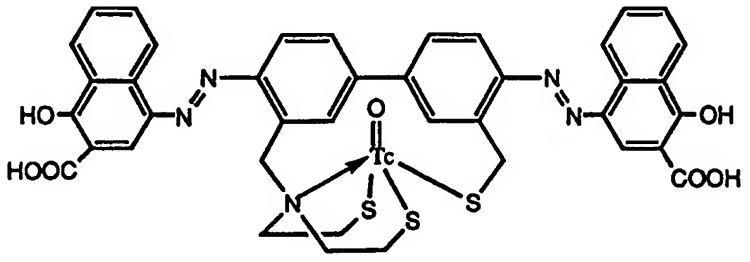
L and L' are -N=N-, -CONH-, -NHCO-, -HN-NH-, or -C=C-, and can be the same or different from each other; and

Ar and Ar' are



and can be the same or different from each other, where each of V, W, Q, Y, Z, A and B is OH, COOH, H, R^5 (R^5 is C_{1-6} hydrocarbon), CO_2R^6 (R^6 is C_{1-6} hydrocarbon), $CONH_2$, CN, NH_2, CH_2NH_2 or SO_3 and can be the same or different from each other.

6. A compound according to claim 5 wherein the formula is selected from the group consisting of:



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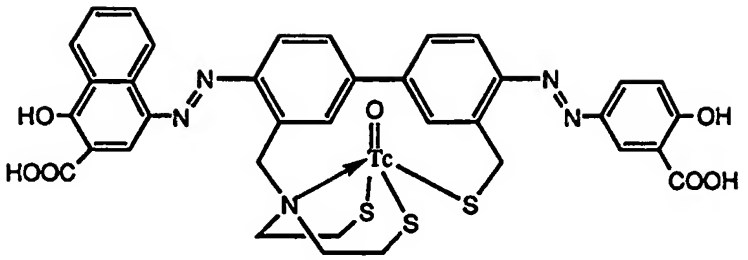

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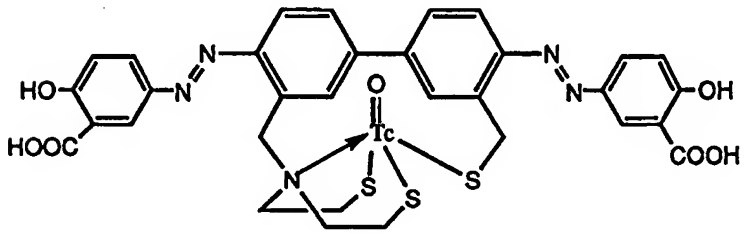

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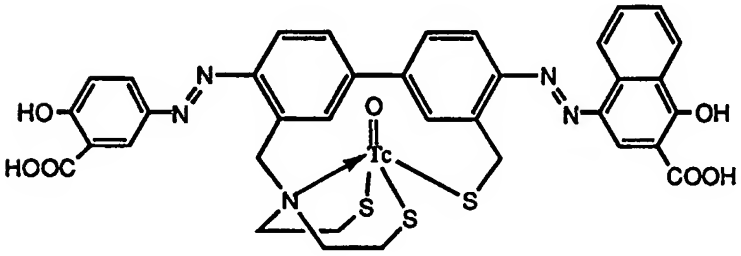
and



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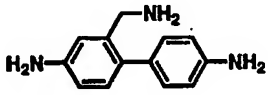
7. A compound of the formula



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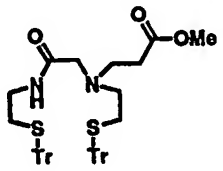
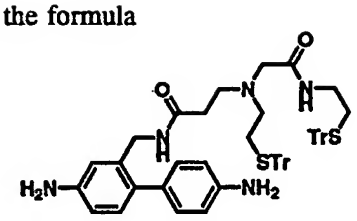
8. A compound of the formula



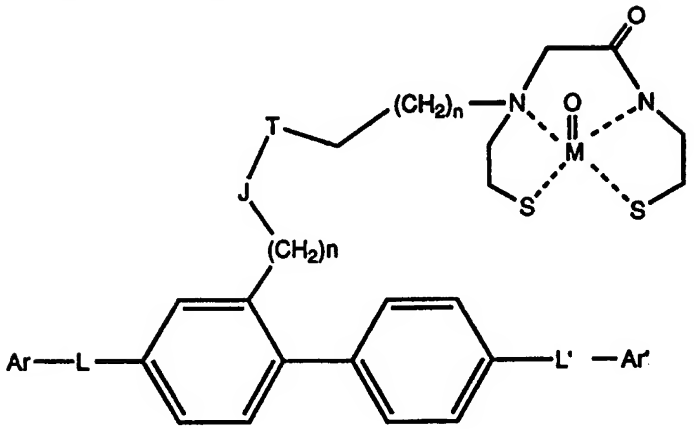
82

9. A compound of the formula



83

10. An amyloid binding compound of the formula



(III)

and pharmaceutically acceptable salts thereof,

wherein

J is NH, O or S;

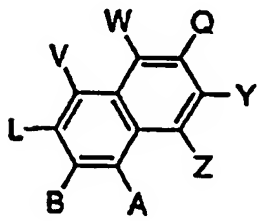
T is CO or CH_2;

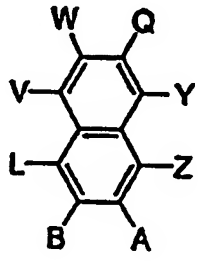
n is the number 1, 2, 3, 4, 5 or 6;

M is ^{99m}Tc, ^{111}In, ^{90}Y, ^{99}Tc or ^{186}Re;

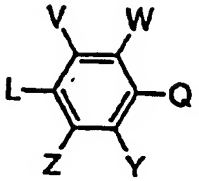
L and L' are -N=N-, -CONH-, -NHCO-, -HN-NH-, or -C=C-, and can be the same or different from each other; and

Ar and Ar' are



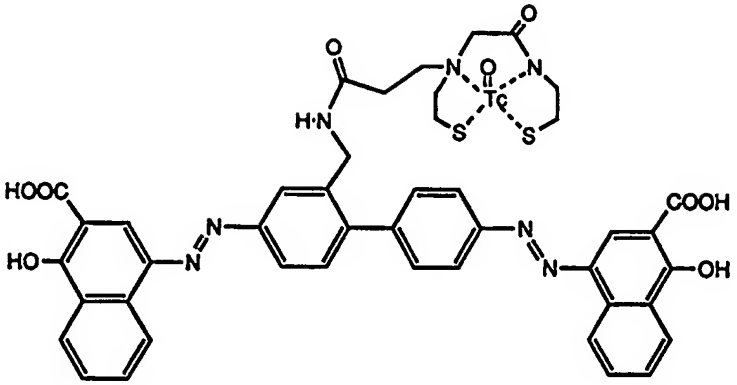


or



and can be the same or different from each other, where each of V, W, Q, Y, Z, A and B is OH, COOH, H, R^5 (R^5 is C_{1-6} hydrocarbon), CO_2R^6 (R^6 is C_{1-6} hydrocarbon), $CONH_2$, CN, NH_2, CH_2NH_2 or SO_3 and can be the same or different from each other.

11. A compound according to claim 10 wherein the formula is selected from the group consisting of:



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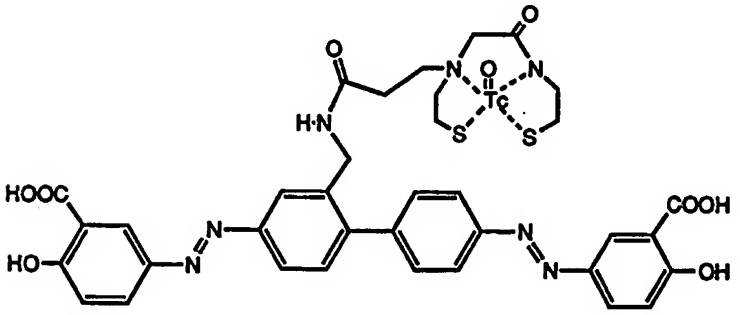

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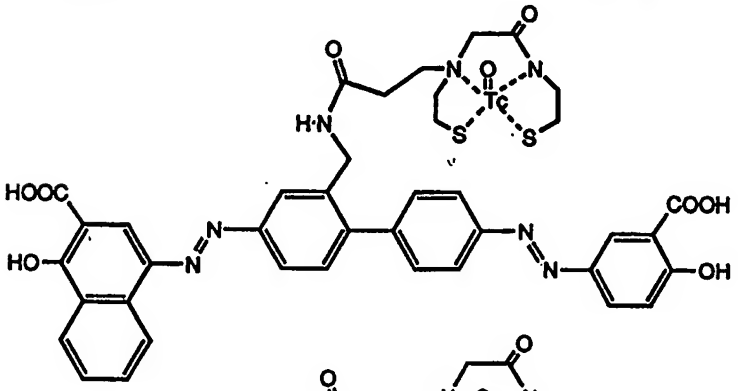
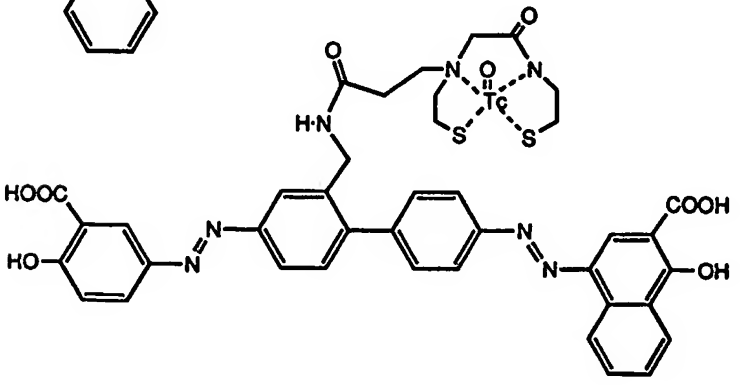


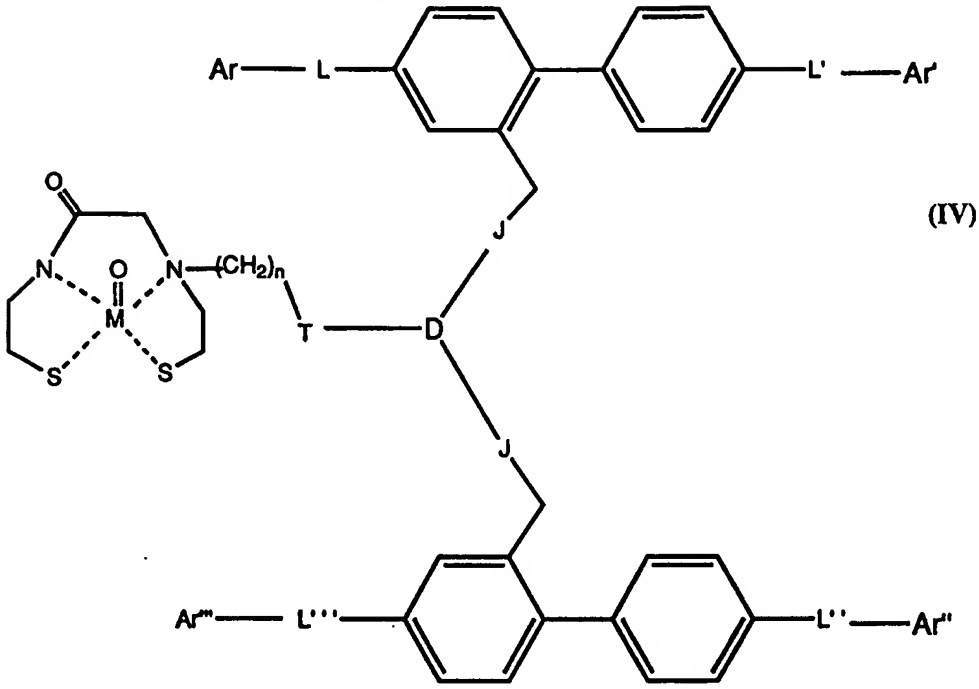
146

and



147

12. An amyloid binding compound of the formula



(IV)

and pharmaceutically acceptable salts thereof,

wherein

J is NH or S;

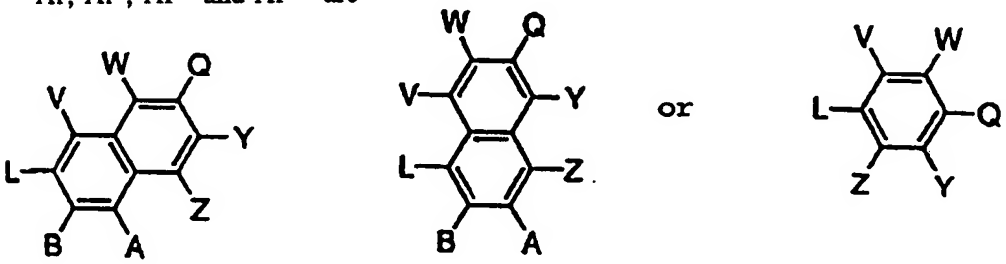
T is CO or CH_2;

n is the number 1, 2, 3, 4, 5 or 6;

M is ^{99m}Tc, ^{111}In, ^{90}Y, ^{99}Tc or ^{186}Re;

L, L', L'' and L''' are -N=N-, -CONH-, -NHCO-, -HN-NH-, or -C=C-, and can be the same or different from each other;

Ar, Ar', Ar'' and Ar''' are



and can be the same or different from each other, where each of V, W, Q, Y, Z, A and B is OH, COOH, H, R^5 (R^5 is C_{1-6} hydrocarbon), CO_2R^6 (R^6 is C_{1-6} hydrocarbon), $CONH_2$, CN, NH_2, CH_2NH_2 or SO_3 and can be the same or different from each other; and

when J is NH and T is CO, then D is a trifunctional linker with two carboxyl groups

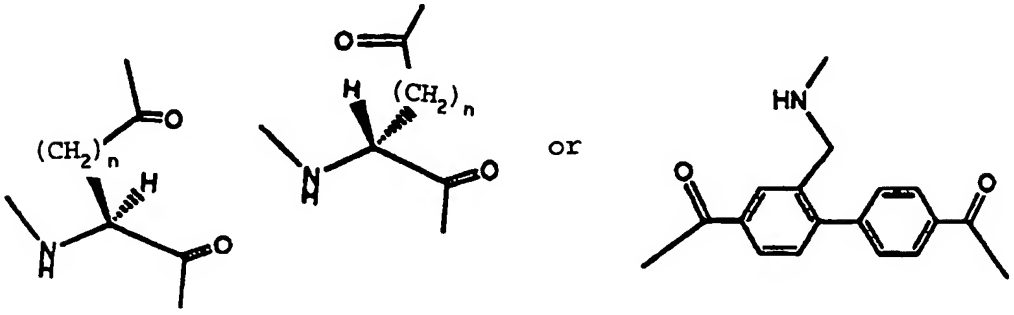
and one amine group,

when J is NH and T is CH_2, then D is $COCH_2(CH_2S)CH_2CO$,

when J is S and T is CO, then D is $CH_2CH(CH_2NH)CH_2$, and

when J is S and T is CH_2, then D is $CH_2CH(CH_2S)CH_2$.

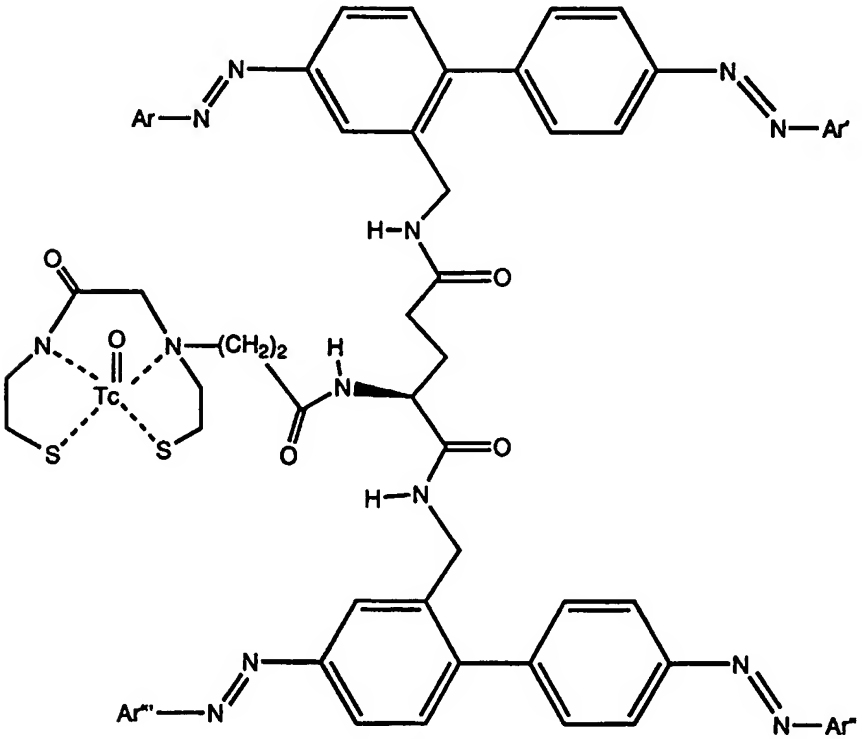
13. The compound according to claim 12 wherein J is NH, T is CO and D is



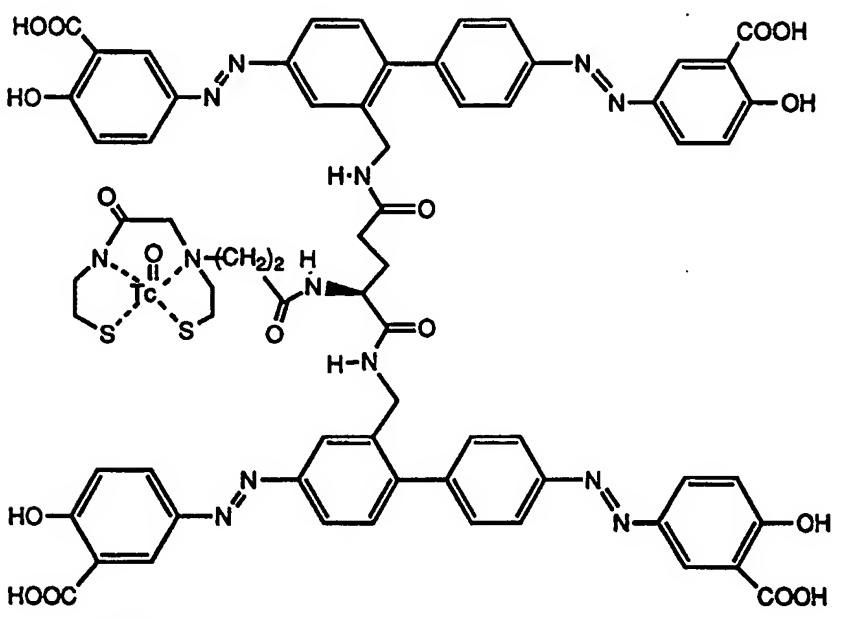
or

and n is the number 1 or 2.

14. The compound according to claim 12 wherein the formula is:



15. The compound according to claim 14 wherein the formula is selected from the group consisting of:



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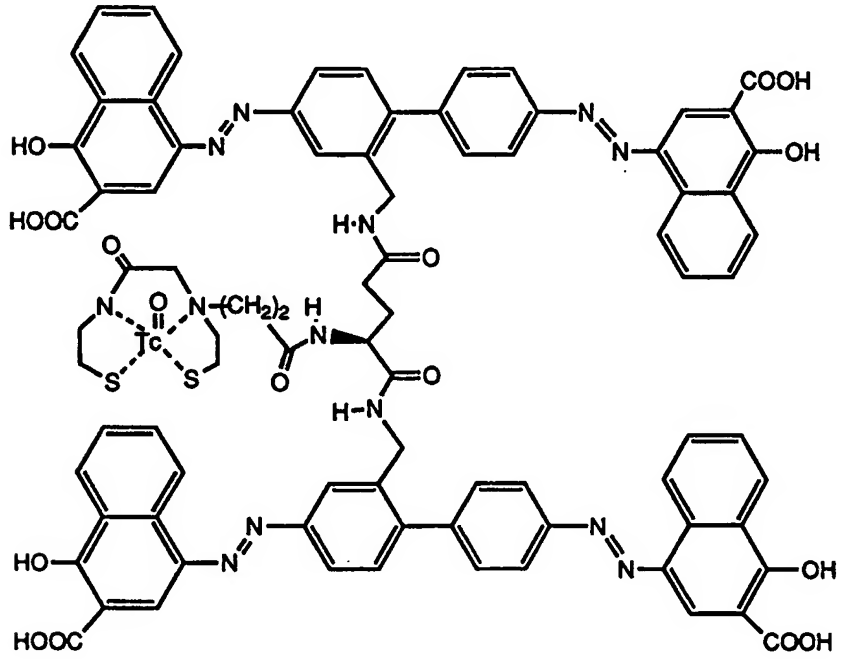
and



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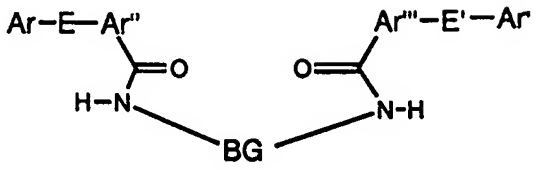
16. An amyloid binding compound of the formula



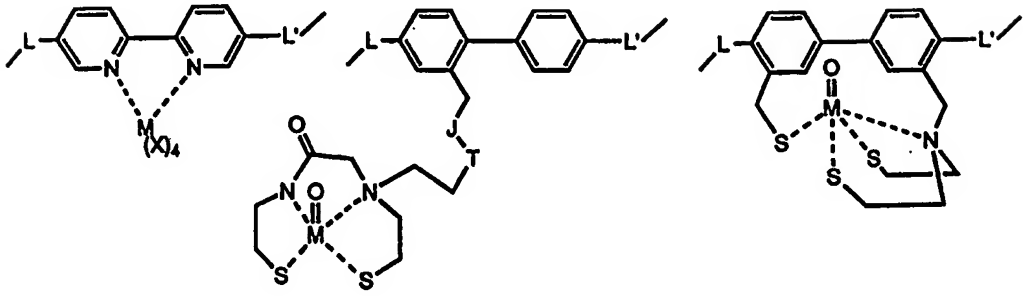
(V)

and pharmaceutically acceptable salts thereof,

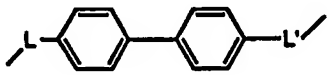
wherein

BG is any dicarbonyl or dithiocarbonyl moiety;

E and E' are



or



and can be the same or different from each other,

wherein

M is ^{99m}Tc, ^{111}In, ^{90}Y, ^{99}Tc or ^{186}Re;

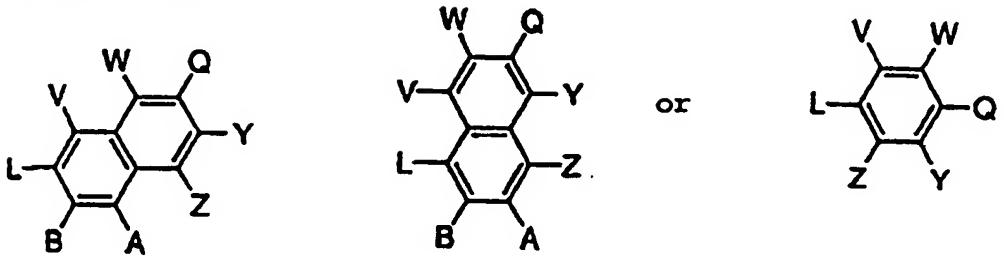
L and L' are -N=N-, -CONH-, -NHCO-, -HN-NH-, or -C=C-, and can be the same or different from each other;

J is NH or S;

T is CO or CH_2;

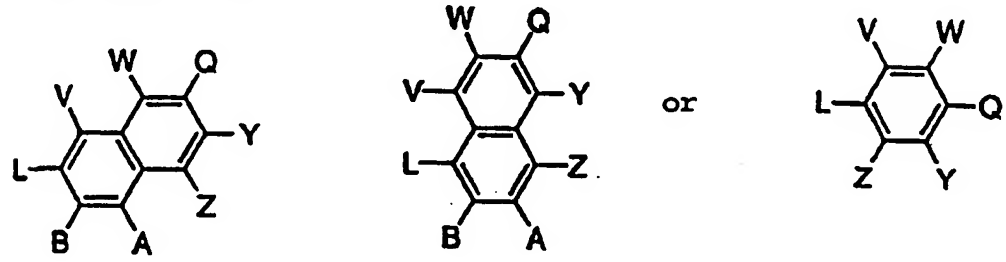
X is Cl, I, Br, F, $P(R^2)_3$ (R^2 is C_{1-6} hydrocarbon), $P(Ar^2)_3$ (Ar^2 is aryl or substituted aryl), R^3NC (R^3 is C_{1-6} hydrocarbon), Ar^3NC (Ar^3 is aryl or substituted aryl), SR^4 (R^4 is CH_2CH_2SH or C_{1-6} hydrocarbon), or $P(R^5)_2R^6$ (R^5 is C_{1-6} hydrocarbon; R^6 is C_{1-6} hydrocarbon or $CH_2CH_2P(CH_3)_2$, and each X can be the same or different from each other;

Ar and Ar' are



and can be the same or different from each other, where each of V, W, Q, Y, Z, A and B is OH, COOH, H, R^5 (R^5 is C_{1-6} hydrocarbon), CO_2R^6 (R^6 is C_{1-6} hydrocarbon), $CONH_2$, CN, NH_2, CH_2NH_2 or SO_3 and can be the same or different from each other, and

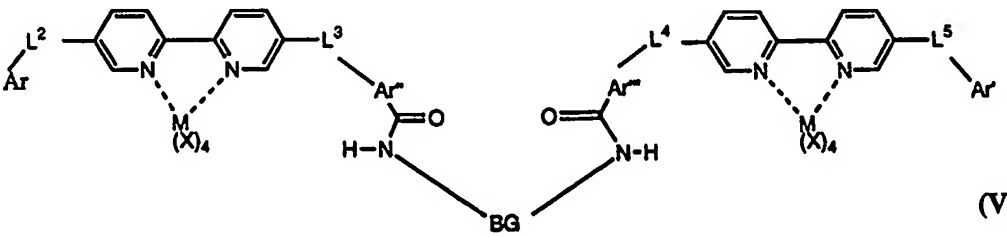
Ar'' and Ar''' are



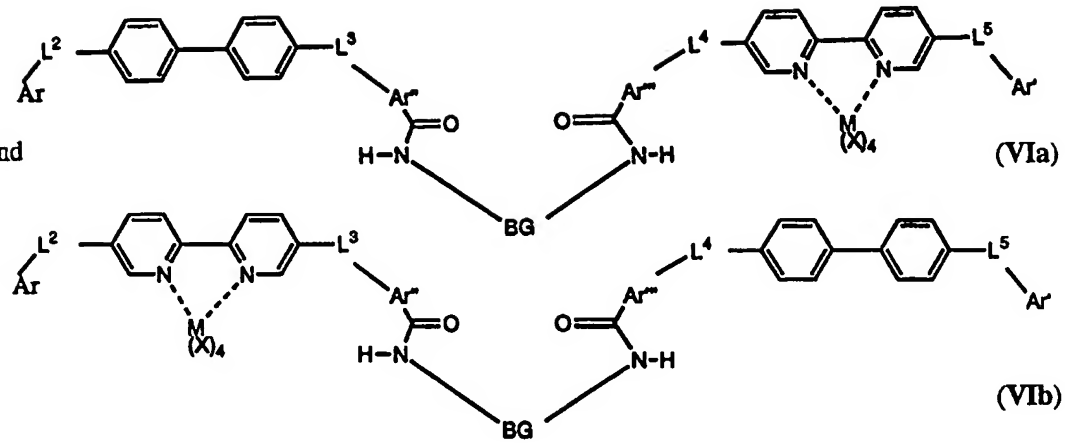
and can be the same or different from each other, where one of V, W, Q, Y, Z, A and B is COOH and each of the others is OH, COOH, H, R^5 (R^5 is C_{1-6} hydrocarbon), CO_2R^6 (R^6 is C_{1-6} hydrocarbon), $CONH_2$, CN, NH_2, CH_2NH_2 or SO_3 and can be the same or different from each other.

17. The amyloid binding compound of claim 16 wherein BG is $CO(CH_2)_nCO$, $CS(CH_2)_nCS$, $COCH(NH_2)(CH_2)_2CO$ or $COCH(NH_2)CH_2CO$, and wherein n is the number 1-6.

18. A compound according to claim 16 wherein the formula is selected from the group consisting of



(VI)

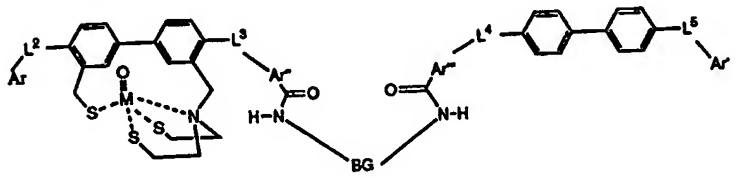


and (VIa)

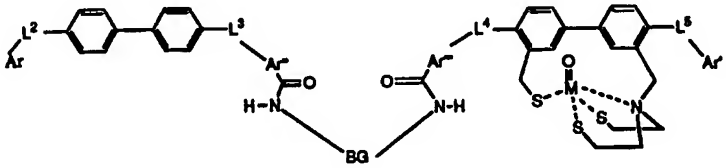
(VIb)

wherein L^2, L^3, L^4 and L^5 are -N=N-, -CONH-, -NHCO-, -HN-NH-, or -C=C-, and can be the same or different from each other.

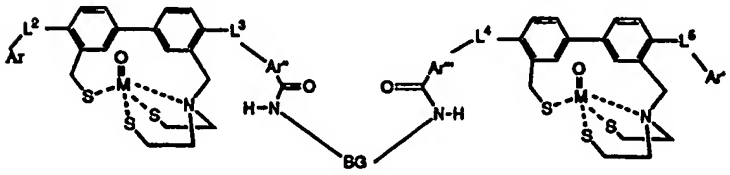
19. A compound according to claim 16 wherein the formula is selected from the group consisting of



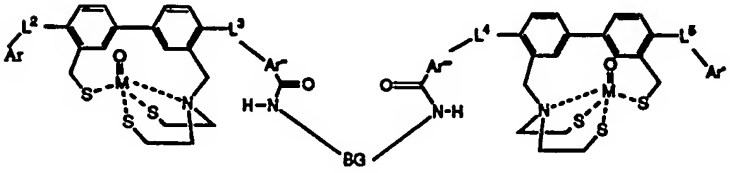
(VII)



(VIIa)

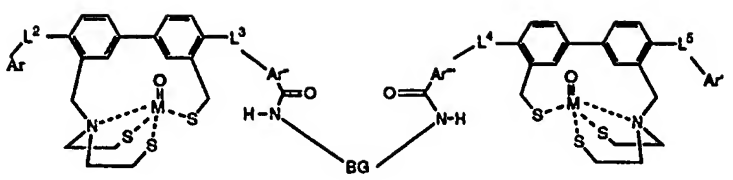


(VIIb)



(VIIc)

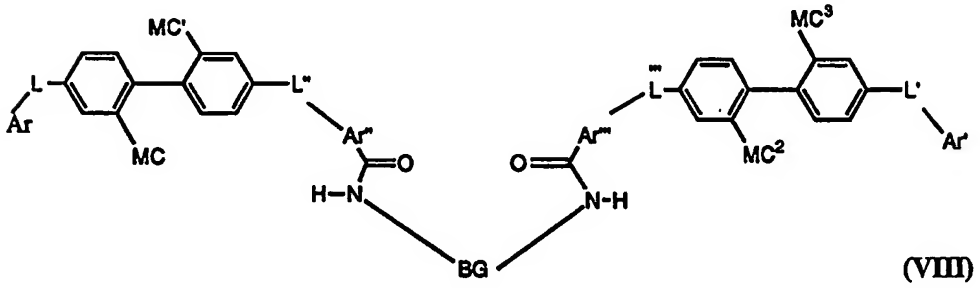
and



(VIId)

wherein L^2, L^3, L^4 and L^5 are -N=N-, -CONH-, -NHCO-, -HN-NH-, or -C=C-, and can be the same or different from each other.

20. A compound according to claim 16 wherein the formula is

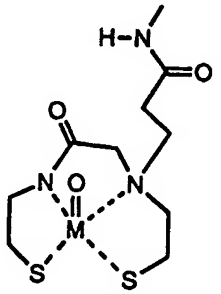


(VIII)

wherein

L^2, L^3, L^4 and L^5 are -N=N-, -CONH-, -NHCO-, -HN-NH-, or -C=C-, and can be the same or different from each other; and

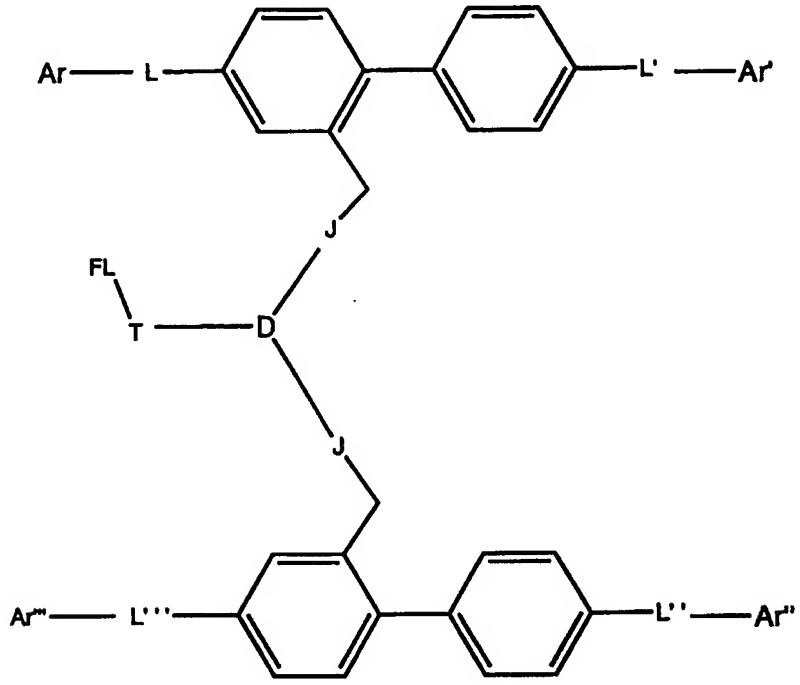
MC and MC^1, MC^2 and MC^3 are



or H

where any one of MC, MC^1, MC^2 or MC^3 is a metal binding group and the others are H, or where MC or MC^1 is H and MC^2 or MC^3 is H and the others are a metal binding group.

21. An amyloid binding compound of the formula



(IX)

and pharmaceutically acceptable salts thereof,

wherein

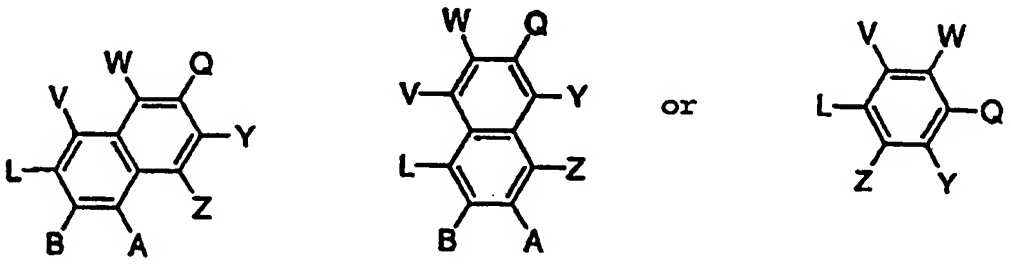
J is NH or S;

T is CO or CO_2;

FL is fluorescein, rhodamine, coumarin or any other fluorescent moiety;

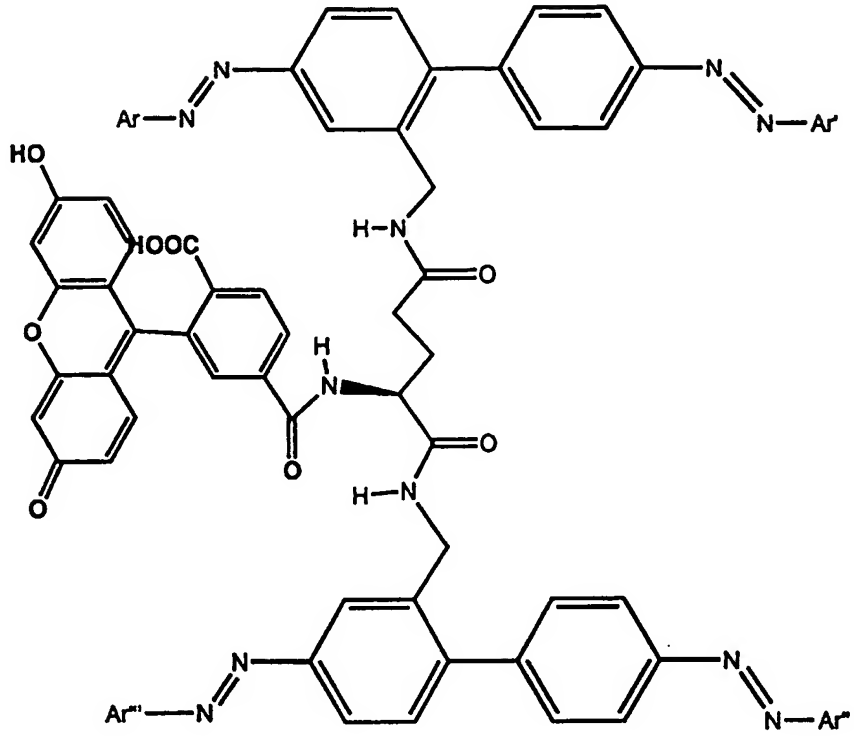
L and L' are -N=N-, -CONH-, -NHCO-, -HN-NH-, or -C=C-, and can be the same or different from each other; and

Ar and Ar' are



and can be the same or different from each other, where each of V, W, Q, Y, Z, A and B is OH, COOH, H, R^5 (R^5 is C_{1-6} hydrocarbon), CO_2R^6 (R^6 is C_{1-6} hydrocarbon), $CONH_2$, CN, NH_2, CH_2NH_2 or SO_3 and can be the same or different from each other.

22. A compound according to claim 21 wherein the formula is



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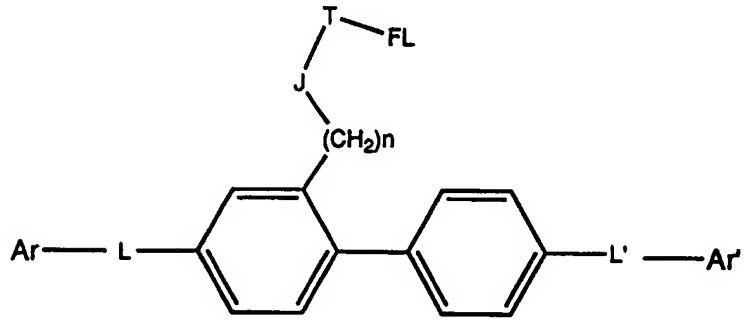
23. An amyloid binding compound of the formula



(X)

and pharmaceutically acceptable salts thereof,

wherein

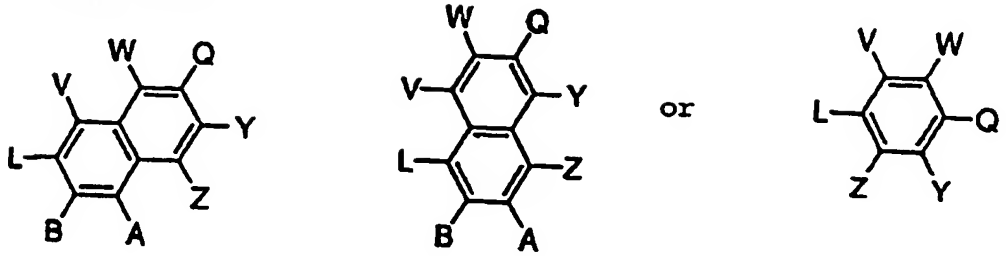
J is NH or S;

T is CO or CO_2;

FL is fluorescein, rhodamine, coumarin or any other fluorescent moiety;

L and L' are -N=N-, -CONH-, -NHCO-, -HN-NH-, or -C=C-, and can be the same or different from each other; and

Ar and Ar' are



and can be the same or different from each other, where each of V, W, Q, Y, Z, A and B is OH, COOH, H, R^5 (R^5 is C_{1-6} hydrocarbon), CO_2R^6 (R^6 is C_{1-6} hydrocarbon), $CONH_2$, CN, NH_2, CH_2NH_2 or SO_3 and can be the same or different from each other.

24. A method for diagnosing the degree of progression of Alzheimer's disease in a mammal, comprising:

providing a first mammal having Alzheimer's disease, said mammal having brain amyloid fibrils;

providing a labeled ligand capable of interacting with said amyloid fibrils;

administering said labeled ligand to said first mammal under conditions which allow said labeled ligand to interact with said amyloid fibrils in said brain so as to result in labeled amyloid fibrils; and

determining the localization or quantification of said labeled amyloid fibrils in said first mammal by imaging so as to diagnose the degree of progression of said Alzheimer's disease.

25. The method of claim 24 wherein said amyloid fibrils are selected from the group consisting of precursor forms of aggregated β-amyloid, intermediate forms of β-amyloid, mature forms of β-amyloid, and combinations thereof.

26. The method of claim 24 wherein said amyloid fibrils are selected from the group consisting of β-amyloid protofibrils, type-1 fibrils, type-2 fibrils, neuritic plaque, diffuse amyloid, and combinations thereof.

27. The method of claim 24 wherein said amyloid fibrils are β-amyloid protofibrils.

28. The method of claim 24 wherein said labeled ligand is an organometallic imaging agent.

29. The method of claim 24 wherein said labeled ligand is an analog of Congo Red or Chrysamine G.

30. The method of claim 24 wherein said labeled ligand is a compound of formula I or a pharmaceutically acceptable salt thereof.

31. The method of claim 24 wherein said labeled ligand is a compound selected from the group consisting of formulas II, III, IV, V and pharmaceutically acceptable salts thereof.

32. The method of claim 24 wherein said labeled ligand is capable of interacting specifically with brain amyloid fibrils.

33. The method of claim 24 wherein said labeled ligand is capable of interacting specifically with β-amyloid protofibrils.

34. The method of claim 24 wherein said labeled ligand is capable of interacting specifically with fibrils selected from the group consisting of type-1 fibrils and type-2 fibrils.

35. The method of claim 24 wherein said labeled ligand is selected from the group consisting of a gamma emitter and a beta emitter.

36. The method of claim 24 wherein said label is selected from the group consisting of technetium-99m, indium-111, yttrium-90 and rhenium-186.

37. The method of claim 24 wherein said imaging is selected from the group consisting of radioimaging, magnetic resonance imaging and single photon emission computed tomographic imaging.

38. The method of claim 24 further comprising comparing said localization or quantification of said labeled amyloid fibrils to a standard, said standard being selected from

the group consisting of a localization or quantification pattern of a second mammal not having Alzheimer's disease, and a localization or quantification pattern obtained from an earlier determination from said first mammal.

39. A method for monitoring the response to a therapy in a mammal having Alzheimer's disease, comprising:

providing a mammal having Alzheimer's disease, said mammal having brain amyloid fibrils;

treating said mammal with a therapy for said Alzheimer's disease;

monitoring the response of said mammal to said treating step by determining whether said therapy alters the localization or quantification of said amyloid fibrils in said mammal.

40. The method of claim 39 wherein said amyloid fibrils are selected from the group consisting of precursor forms of aggregated β-amyloid, intermediate forms of β-amyloid, mature forms of β-amyloid, and combinations thereof.

41. The method of claim 39 wherein said amyloid fibrils are selected from the group consisting of β-amyloid protofibrils, type-1 fibrils, type-2 fibrils, neuritic plaque, diffuse amyloid, and combinations thereof.

42. The method of claim 39 wherein said amyloid fibrils are β-amyloid protofibrils.

43. The method of claim 39 wherein said determining comprises:

providing a labeled ligand capable of interacting with said amyloid fibrils;

administering said labeled ligand to said mammal under conditions which allow said labeled ligand to interact with said amyloid fibrils in said brain so as to result in labeled amyloid fibrils; and

determining the localization or quantification of said labeled amyloid fibrils in said mammal by imaging.

44. The method of claim 43 wherein said labeled ligand is a compound of formula I or a pharmaceutically acceptable salt thereof.

45. The method of claim 43 wherein said labeled ligand is a compounds selected from the group consisting of formulas **II**, **III**, **IV**, **V** and pharmaceutically acceptable salts thereof.

46. A method for evaluating the ability of an agent to alter the localization or quantification of brain amyloid fibrils in a mammal, comprising:

providing a mammal having brain amyloid fibrils;

providing an agent;

administering said agent to said mammal; and

determining whether said agent alters the localization or quantification of such brain amyloid fibrils in said mammal.

47. The method of claim 46 wherein said amyloid fibrils are selected from the group consisting of precursor forms of aggregated β-amyloid, intermediate forms of β-amyloid, mature forms of β-amyloid, and combinations thereof.

48. The method of claim 46 wherein said amyloid fibrils are selected from the group consisting of β-amyloid protofibrils, type-1 fibrils, type-2 fibrils, neuritic plaque, diffuse amyloid, and combinations thereof.

49. The method of claim 46 wherein said amyloid fibrils are β-amyloid protofibrils.

50. The method of claim 46 wherein said determining comprises:

providing a labeled ligand capable of interacting with said amyloid fibrils;

administering said labeled ligand to said mammal under conditions which allow said labeled ligand to interact with said amyloid fibrils in said brain so as to result in labeled amyloid fibrils; and

determining the localization or quantification of said labeled amyloid fibrils in said mammal by imaging.

51. The method of claim 50 wherein said labeled ligand is a compound of formula I or a pharmaceutically acceptable salt thereof.

52. The method of claim 50 wherein said labeled ligand is a compound selected from the group consisting of formulas **II**, **III**, **IV**, **V** and pharmaceutically acceptable salts thereof.

53. A method for identifying an agent useful for treating a mammal having a disease associated with aggregated amyloid, comprising:

providing a mammal having a disease associated with aggregated amyloid, said mammal having amyloid fibrils;

providing an agent;

administering said agent to said mammal; and

determining if said agent alters the localization or quantification of said amyloid fibrils in said mammal, an alteration in said localization or quantification which results in a localization or quantification more similar to that of a mammal which does not have said disease being correlated with said agent being useful for treating said mammal having said disease.

54. The method of claim 53 wherein said disease is selected from the group consisting of Alzheimer's disease, type II diabetes, B-cell lymphoma, Creutzfeldt-Jacob disease, bovine spongiform encephalopathy, familial transthyretin amyloidosis, complications from dialysis, and serum amyloid A systemic amyloidosis.

55. The method of claim 54 wherein said disease is Alzheimer's disease.

56. The method of claim 53 wherein said amyloid fibrils are selected from the group consisting of precursor forms of aggregated β-amyloid, intermediate forms of β-amyloid, mature forms of β-amyloid, and combinations thereof.

57. The method of claim 53 wherein said amyloid fibrils are selected from the group consisting of β-amyloid protofibrils, type-1 fibrils, type-2 fibrils, neuritic plaque, diffuse amyloid, and combinations thereof.

58. The method of claim 53 wherein said amyloid fibrils are β-amyloid protofibrils.

59. The method of claim 53 wherein said determining comprises:

providing a labeled ligand capable of interacting with said amyloid fibrils;

administering said labeled ligand to said mammal under conditions which allow said labeled ligand to interact with said amyloid fibrils in said brain so as to result in labeled amyloid fibrils; and

determining the localization or quantification of said labeled amyloid fibrils in said mammal by imaging.

60. The method of claim 59 wherein said labeled ligand is a compound of formula I or a pharmaceutically acceptable salt thereof.

61. The method of claim 59 wherein said labeled ligand is a compound selected from the group consisting of formulas **II**, **III**, **IV**, **V** and pharmaceutically acceptable salts thereof.

62. The agent obtainable by the method of claim 53.

63. A method for determining the localization or quantification of amyloid fibrils in a mammal, comprising:

providing a mammal having amyloid fibrils;

providing an organometallic ligand capable of interacting with said amyloid fibrils;

administering said organometallic ligand to said mammal under conditions which allow said organometallic ligand to interact with said amyloid fibrils so as to result in organometallic ligand-amyloid fibril complexes; and

determining the localization or quantification of said organometallic ligand-amyloid fibril complexes in said mammal.

64. The method of claim 63 wherein said amyloid fibrils are selected from the group consisting of precursor forms of aggregated β-amyloid, intermediate forms of β-amyloid, mature forms of β-amyloid, and combinations thereof.

65. The method of claim 63 wherein said amyloid fibrils are selected from the group consisting of β-amyloid protofibrils, type-1 fibrils, type-2 fibrils, neuritic plaque, diffuse amyloid, and combinations thereof.

66. The method of claim 63 wherein said amyloid fibrils are β-amyloid protofibrils.

67. The method of claim 63 wherein said determining comprises:

providing a labeled ligand capable of interacting with said amyloid fibrils;

administering said labeled ligand to said mammal under conditions which allow said labeled ligand to interact with said amyloid fibrils in said brain so as to result in labeled amyloid fibrils; and

determining the localization or quantification of said labeled amyloid fibrils in said mammal by imaging.

68. The method of claim 67 when said labeled ligand is a compound of formula I or a pharmaceutically acceptable salt thereof.

69. The method of claim 67 wherein said labeled ligand is a compound selected from the group consisting of formulas II, III, IV, V and pharmaceutically acceptable salts thereof.

70. The method of claim 63 wherein said amyloid fibrils are in a portion of the body of said mammal selected from the group consisting of the brain, pancreas, vasculature, spleen, liver, kidneys, adrenals, lymph nodes, muscle, cardiovascular system, skin and any combination thereof.

71. The method of claim 63 wherein said determining step is by imaging.

72. The method of claim 63 wherein said administering and said determining steps are repeated after a time interval so as to establish a time course for said localization or said quantification of said organometallic ligand-amyloid fibril complexes in said mammal.

73. The method of claim 63 wherein said mammal is deceased and said administering step is to the postmortem brain or a portion thereof of said deceased mammal.

74. The method of claim 73 wherein said determining step is by the process selected from the group consisting of autoradiography, SPECT, PET and magnetic resonance imaging.

75. A method for treating Alzheimer's disease in a mammal, comprising:

providing a mammal having Alzheimer's disease, said mammal having non-aggregated amyloid proteins or aggregated amyloid proteins, or combinations thereof;

providing an organometallic ligand capable of interacting with said non-aggregated amyloid proteins or with said aggregated amyloid proteins or with both said amyloid proteins;

administering a therapeutically effective amount of said organometallic ligand to said mammal under conditions which allow said organometallic ligand to interact with said non-aggrgated amyloid proteins or with said aggregated amyloid proteins or with both said amyloid proteins, so as to inhibit aggregation of said amyloid proteins such that treatment of said Alzheimer's disease occurs.

76. The method of claim 75 wherein said aggregated amyloid proteins are Alzheimer's disease associated β-amyloid fibrils.

77. The method of claim 75 wherein said aggregated amyloid proteins are selected from the group consisting of protofibrils, type-1 fibrils, type-2 fibrils, neuritic plaque, diffuse amyloid and combinations thereof.

78. The method of claim 75 wherein said organometallic ligand interacts specifically with β-amyloid protofibrils.

79. The method of claim 75 wherein said organometallic ligand is a compound of formula I or a pharmaceutically acceptable salt thereof.

80. The method of claim 75 wherein said labeled ligand is a compound selected from the group consisting of formulas II, III, IV, V and pharmaceutically acceptable salts thereof.

81. The method of claim 75 wherein the metal in said organometallic ligand is selected from the group consisting of Cd, Zn, Co, Cu, Fe, Ni, an oxo form of these metals, and combinations thereof.

82. A pharmaceutical composition for use in treating Alzheimer's disease in a

mammal, comprising:

a therapeutically effective amount of an organometallic ligand, said ligand being able to interact with amyloid proteins in a mammal in need of treatment for Alzheimer's disease; and

a pharmaceutically acceptable carrier.

83. The method of claim 82 wherein said amyloid proteins are selected from the group consisting of precursor forms of aggregated β-amyloid, intermediate forms of β-amyloid, mature forms of β-amyloid, and combinations thereof.

84. The method of claim 82 wherein said amyloid proteins are selected from the group consisting of β-amyloid protofibrils, type-1 fibrils, type-2 fibrils, neuritic plaque, diffuse amyloid, and combinations thereof.

85. The method of claim 82 wherein said amyloid proteins are β-amyloid protofibrils.

86. The pharmaceutical composition of claim 82 wherein said organometallic ligand is a compound of formula I or a pharmaceutically acceptable salt thereof.

87. The method of claim 82 wherein said labeled ligand is a compound selected from the group consisting of formulas II, III, IV, V and pharmaceutically acceptable salts thereof.

88. A method for determining the localization or quantification of amyloid fibrils in a deceased mammal, comprising:

providing a deceased mammal or a portion thereof having amyloid fibrils;

providing an organometallic ligand capable of interacting with said amyloid fibrils;

administering said organometallic ligand to said deceased mammal or portion thereof under conditions which allow said organometallic ligand to interact with said amyloid fibrils so as to result in organometallic ligand-amyloid fibril complexes; and

determining the localization or quantification of said complexes in said mammal or portion thereof.

89. The method of claim 88 wherein said amyloid fibrils are selected from the group consisting of precursor forms of aggregated β-amyloid, intermediate forms of β-amyloid, mature forms of β-amyloid, and combinations thereof.

90. The method of claim 88 wherein said amyloid fibrils are selected from the group consisting of β-amyloid protofibrils, type-1 fibrils, type-2 fibrils, neuritic plaque, diffuse amyloid, and combinations thereof.

91. The method of claim 88 wherein said amyloid fibrils are β-amyloid protofibrils.

92. The method of claim 88 wherein said organometallic ligand is a compound of formula **I** or a pharmaceutically acceptable salt thereof.

93. The method of claim 88 wherein said labeled ligand is a compound selected from the group consisting of formulas **II**, **III**, **IV**, **V** and pharmaceutically acceptable salts thereof.

94. A method for detecting the presence of aggregated prion protein in a mammal, comprising:

providing a mammal;

providing bodily fluid or tissue obtained from said mammal;

providing a labeled ligand capable of interacting with aggregated prion protein;

contacting said bodily fluid or tissue *in vitro* with said labeled ligand under conditions which allow said labeled ligand to interact with said aggregated prion protein if said aggregated prion protein is present in said bodily fluid or tissue, so as to result in labeled aggregated prion protein; and

determining the presence or absence of said labeled aggregated prion protein in said bodily fluid or tissue.

95. The method of claim 94 wherein said mammal has a prion disease selected from the group consisting of scrapie, bovine spongiform encephalopathy and Creutzfeldt-Jacob disease.

96. The method of claim 94 wherein said bodily fluid is lymph.

97. The method of claim 94 wherein said labeled ligand is a compound of formula I or a pharmaceutically acceptable salt thereof.

98. The method of claim 94 wherein said labeled ligand is a compound selected from the group consisting of formulas II, III, IV, V and pharmaceutically acceptable salts thereof.

99. A method for detecting the presence of aggregated prion protein in a mammal, comprising:

providing a mammal;

providing a labeled ligand capable of interacting with aggregated prion protein;

administering said labeled ligand to said mammal under conditions which allow said labeled ligand to interact with said aggregated prion protein if said aggregated prion protein is present in said mammal, so as to result in labeled aggregated prion protein; and

determining the presence or absence of said labeled aggregated prion protein in said mammal by imaging.

100. A method for determining the presence of aggregated intracellular β-amyloid, comprising:

providing cells having β-amyloid;

providing a fluorescent ligand capable of interacting with aggregated β-amyloid;

contacting said cells with said fluorescent ligand under conditions which allow said fluorescent ligand to interact with aggregated β-amyloid if aggregated β-amyloid is present so as to result in fluorescent-labeled aggregated β-amyloid; and

determining the presence or absence of a fluorescent signal, the presence of a fluorescent signal indicating the presence of aggregated intracellular β-amyloid.

101. The method of claim 100 wherein said cells are permeabilized prior to contacting said cells with said fluorescent ligand.

102. The method of claim 100 wherein said fluorescent ligand is a compound selected from the group consisting of formula IX, formula X, and pharmaceutically acceptable salts thereof.

103. The method of claim 100 wherein said fluorescent ligand is selected from the group consisting of formula **131**, **133**, **135**, and pharmaceutically acceptable salts thereof.

104. The method of claim 100 wherein said cells are from a cell culture.

105. A method for identifying an agent useful for treating a mammal for a disease characterized by aggregated intracellular β-amyloid, comprising:

providing cells having β-amyloid;

providing an agent;

providing a fluorescent ligand capable of interacting with β-amyloid fibrils;

contacting said cells with said agent to form a mixture under conditions which allow aggregation of said β-amyloid if said agent was not present;

contacting said mixture with said fluorescent ligand under conditions which allow said fluorescent ligand to interact with β-amyloid fibrils if said β-amyloid fibrils are present so as to result in fluorescent-labeled β-amyloid fibrils;

determining if said agent inhibits aggregation of said β-amyloid, the presence of a fluorescent signal indicating the presence of β-amyloid fibrils and therefore minimal or no inhibition by said agent, and the absence of a fluorescent signal indicating the absence of β-amyloid fibrils and therefore inhibition by said agent, the inhibition being correlated with said agent being useful for treating a mammal for a disease characterized by aggregated intracellular β-amyloid.

106. The method of claim 105 wherein said disease is selected from the group consisting of Down's syndrome and Alzheimer's disease.

107. The method of claim 105 wherein said cells are neurons.

108. The method of claim 105 wherein said fluorescent ligand is a compound selected from the group consisting of formula **IX**, formula **X**, and pharmaceutically acceptable salts thereof.

109. The method of claim 105 wherein said fluorescent ligand is selected from the group consisting of formula **131**, **133**, **135**, and pharmaceutically acceptable salts thereof.

110. A method for identifying a labeled ligand which selectively binds to one type of β-amyloid fibril, comprising:

providing a labeled compound;

providing first β-amyloid fibrils;

providing second β-amyloid fibrils;

contacting said labeled compound with said first β-amyloid fibrils under conditions which allow said labeled compound to interact with said first β-amyloid fibrils;

determining if said labeled compound binds to said first β-amyloid fibrils, and if said labeled compound does not bind to said first β-amyloid fibrils,

contacting said labeled compound with said second β-amyloid fibrils under conditions which allow said labeled compound to interact with said second β-amyloid fibrils; and

determining if said labeled compound binds to said second β-amyloid fibrils, binding being correlated with a labeled ligand which selectively binds to said second β-amyloid fibrils as compared to said first β-amyloid fibrils.

111. The method of claim 110 wherein said first β-amyloid fibrils are selected from the group consisting of protofibrils, type-1 fibrils, type-2 fibrils, neuritic plaque and diffuse plaque.

112. The method of claim 110 wherein said second β-amyloid fibrils are selected from the group consisting of protofibrils, type-1 fibrils, type-2 fibrils, neuritic plaque and diffuse plaque.

113. The method of claim 110 wherein said second β-amyloid fibrils are protofibrils.

114. The method of claim 110 wherein said labeled ligand is a compound of formula I or a pharmaceutically acceptable salt thereof.

115. The method of claim 110 wherein said labeled ligand is a compound selected from the group consisting of formulas II, III, IV, V and pharmaceutically acceptable salts thereof.

116. The labeled ligand identified in accordance with claim 110.

AMENDED CLAIMS

[received by the International Bureau on 12 November 1997 (12.11.97);
original claims 83-85 and 87 amended; remaining claims unchanged (1 page)]

mammal, comprising:

a therapeutically effective amount of an organometallic ligand, said ligand being able to interact with amyloid proteins in a mammal in need of treatment for Alzheimer's disease; and

a pharmaceutically acceptable carrier.

83. The pharmaceutical composition of claim 82 wherein said amyloid proteins are selected from the group consisting of precursor forms of aggregated β-amyloid, intermediate forms of β-amyloid, mature forms of β-amyloid, and combinations thereof.

84. The pharmaceutical composition of claim 82 wherein said amyloid proteins are selected from the group consisting of β-amyloid protofibrils, type-1 fibrils, type-2 fibrils, neuritic plaque, diffuse amyloid, and combinations thereof.

85. The pharmaceutical composition of claim 82 wherein said amyloid proteins are β-amyloid protofibrils.

86. The pharmaceutical composition of claim 82 wherein said organometallic ligand is a compound of formula **I** or a pharmaceutically acceptable salt thereof.

87. The pharmaceutical composition of claim 82 wherein said labeled ligand is a compound selected from the group consisting of formulas **II**, **III**, **IV**, **V** and pharmaceutically acceptable salts thereof.

88. A method for determining the localization or quantification of amyloid fibrils in a deceased mammal, comprising:

providing a deceased mammal or a portion thereof having amyloid fibrils;

providing an organometallic ligand capable of interacting with said amyloid fibrils;

administering said organometallic ligand to said deceased mammal or portion thereof under conditions which allow said organometallic ligand to interact with said amyloid fibrils so as to result in organometallic ligand-amyloid fibril complexes; and

determining the localization or quantification of said complexes in said mammal or portion thereof.

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US97/07792

A. CLASSIFICATION OF SUBJECT MATTER

IPC(6) :Please See Extra Sheet.
US CL :Please See Extra Sheet.
According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

U.S. : 514/353,357,613,647; 534/14; 556/45,110,118, 138;424/1.65,9.4,9.42

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)

Please See Extra Sheet.

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X,P	HAN et al. 'Technetium Complexes for the Quantitation of Brain Amyloid'. J. Am. Chem. Soc., May 1996, Vol. 118, pages 4506-4507. See entire document.	1-4,24-81, 88-104
X,P	Database Caplus on STN, Chemical Abstracts,(Columbus OH,USA) No 125:109277, ASHBURN T.T.et al. 'Amyloid probes based on Congo Red distinguish between fibrils comprising differenct peptides' abstract Chem. Biol., March 1996.	1-29, 32-39, 63-68,70-74, 88-91, 94-96

[X] Further documents are listed in the continuation of Box C. [ ] See patent family annex.

*	Special categories of cited documents:	"T"	later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention
"A"	document defining the general state of the art which is not considered to be of particular relevance	"X"	document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone
"E"	earlier document published on or after the international filing date	"Y"	document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art
"L"	document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)	"&"	document member of the same patent family
"O"	document referring to an oral disclosure, use, exhibition or other means		
"P"	document published prior to the international filing date but later than the priority date claimed		

Date of the actual completion of the international search 19 AUGUST 1997	Date of mailing of the international search report 15 SEP 1997
Name and mailing address of the ISA/US Commissioner of Patents and Trademarks Box PCT Washington, D.C. 20231 Facsimile No. (703) 305-3230	Authorized officer JEAN F. VOLLANO Telephone No. (703) 308-1235

Form PCT/ISA/210 (second sheet)(July 1992)*

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US97/07792

C (Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
A	AWAD et al. 'Synthesis and Antimicrobial Activity of Some New 3-Azo(P-substituted Benzenesulphonamido-)-Bipyridyls and Their Chelates". Synth. React. Inorg. Met.-Org. Chem. March 1991, Vol. 21, No. 3, pages 375-383. See entire document especially page 377.	1
Y	POLLACK et al. 'Sulfonated dyes attenuate the toxic effects of beta-amyloid in a structure-specific fashion'. Neuroscience Letters. March 1995, Vol. 197, pages 211-214.	24-29, 32-34, 46-49
Y,P	W0 96/34853 A1 (UNIVERSITY OF PITTSBURGH) 07 November 1996, see entire document.	46-49, 53-59
Y	CAŬGHEY et al. 'Sulfated Polyanion Inhibition of Scrapie-Associated PrP Accumulation in Cultureed Cells'. Journal of Virology. February 1993, Vol. 67, No. 2, pages 643-650.	99
A,P	WO 95/20979 A1 (THE PICOWER INSTITUTE FOR MEDICAL RESEARCH) 10 August 1995, see entire document.	1-9
Y	KLUNK et al. 'Development of Small Molecule Probes for the Beta-Amyloid Protein of Alzheimer's Disease', Neurobiology of Aging. August 1994, Vol. 15, No. 6, pages 691-698. See entire document.	24-29, 39-43

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US97/07792

Box I Observations where certain claims were found unsearchable (Continuation of item 1 of first sheet)

This international report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons:

1. ☐ Claims Nos.:
because they relate to subject matter not required to be searched by this Authority, namely:

2. ☒ Claims Nos.: 83-85 and 87
because they relate to parts of the international application that do not comply with the prescribed requirements to such an extent that no meaningful international search can be carried out, specifically:

Independent claim 82 is drawn to a composition, dependent claims 83-85 and 87 are drawn to a "method of claim 82". This is unclear as to what a method of claim 82 means and therefore the metes and bounds of the claim are vague and indefinite.

3. ☐ Claims Nos.:
because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

Box II Observations where unity of invention is lacking (Continuation of item 2 of first sheet)

This International Searching Authority found multiple inventions in this international application, as follows:

1. ☐ As all required additional search fees were timely paid by the applicant, this international search report covers all searchable claims.

2. ☐ As all searchable claims could be searched without effort justifying an additional fee, this Authority did not invite payment of any additional fee.

3. ☐ As only some of the required additional search fees were timely paid by the applicant, this international search report covers only those claims for which fees were paid, specifically claims Nos.:

4. ☐ No required additional search fees were timely paid by the applicant. Consequently, this international search report is restricted to the invention first mentioned in the claims; it is covered by claims Nos.:

Remark on Protest

☐ The additional search fees were accompanied by the applicant's protest.

☐ No protest accompanied the payment of additional search fees.

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US97/07792

A. CLASSIFICATION OF SUBJECT MATTER:
IPC (6):

A61K 31/44, 31/165, 31/135, 51/00; C07F 13/00, 1/08, 3/06, 3/08, 15/00

A. CLASSIFICATION OF SUBJECT MATTER:
US CL :

514/353, 357, 613, 647; 534/14; 556/45, 110, 118, 138; 424/1.65, 9.4, 9.42

B. FIELDS SEARCHED
Electronic data bases consulted (Name of data base and where practicable terms used):

APS, CAS ONLINE, CAPLUS

search terms: amyloid, fibrils, alzheimers, imaging, radioactive, technetium, plaque.